Hybridisation, and the Conservation of the Grey Duck in New Zealand

Wiebke Muller

Supervisors:

Dr. James Briskie

Dr. Neil Gemmell

Dedication

To my mother, who, ravaged by dementia, might no longer be able to grasp what the completion of my thesis signifies, but who resolved after her diagnosis that her illness would never restrict the options and choices in education and life of either my brother or myself; and to my father, who proudly supported that resolution every step of the way.

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Abstract

Hybridisation is increasingly acknowledged as a conservation problem. The widespread hybridisation between grey duck (*Anas superciliosa*) and mallard (*A. platyrhynchos*) in New Zealand is a good example of a native species hybridising with a foreign one, and forms the main focus of this thesis. Mallards were introduced into New Zealand from Europe, and hybrids were soon observed. I surveyed the extent of the hybridisation on the West Coast of the South Island and found that, based on phenotype, at least half of population is now hybrids. Mallards and mallard-like hybrids dominate in the eastern South Island, while grey ducks occur only in some areas of the West Coast. Comparison with historical data suggests that the decline of the grey duck and the spread of hybrids has not stabilised and is ongoing. Contrary to expectations most grey ducks were found on agricultural land and most mallards on natural lakes or rivers, so grey ducks probably do not have an advantage over mallards on the less developed West Coast. An alternative theory is proposed here that explains the spatial distribution of hybridisation as a reflection of a temporal pattern. As mallards were first released in the east, the delay taken to cross the Southern Alps could also explain the pattern observed. This hypothesis suggests that the grey duck will persist in the southern West Coast. An analysis of the phenotypes of partners in pairs suggests that mating is positively assortative within each species and within hybrids. In fact, not a single pair of pure grey duck mated with pure mallard was observed in almost a thousand pairs, raising the question of how hybridisation started. There was a tendency for males to be more mallard-like in phenotype than their partners, suggesting there might be a selective advantage to the mallard male phenotype. This may be one factor explaining the

dominance of mallards in the hybrid swarm. To analyse hybridisation at the genetic level, I analysed samples from grey ducks, mallards and domestic ducks with 11 microsatellite loci. This genotyping profile was then compared to ducks captured and shot in New Zealand. Genetic analysis confirms that the ducks in New Zealand were almost exclusively of hybrid origin. Phenotypic hybrid scores correlated with the established genotypic scores, but the correlation was imperfect, suggesting inaccuracies in either or both measures. As the spread of hybrids might be due to the differences in their fitness relative to either parent species, I compared the relative fitness of hybrid ducks using a range of health-related measures such as ecto- and endoparasite loads, immunocompetence, body condition, and heterophil to lymphocyte ratios. Overall, I found no conclusive evidence for any differences between grey duck-like and mallard-like individuals. However, as my sample consisted nearly entirely of hybrids, it is possible that fitness may differ from the parental species. To understand the outcome of hybridisation between two species, I next constructed a mathematical model to simulate hybridisation, and which allowed the specification of parameters describing mating patterns, differential survival, and differential reproductive output. The model successfully predicted the outcomes of two known hybridisation cases. In a sensitivity analysis for mallard and grey duck, the model predicted that this species pair is likely to hybridise under any set of conditions likely to be encountered across their shared range. Finally, in a study within the more general context of hybridisation, the influence of inbreeding on hybridisation rates was investigated using inbred and outbred lines of *Drosophila* species. I found evidence for increased hybridisation in inbred lines, and although further studies are needed to confirm the generality of this pattern, my results

have implications for the management of hybridisation, and for the use of hybridisation as an adaptive strategy. In conclusion, my work suggests it is very likely that the grey duck will become extinct as a separate species in New Zealand in the near future, and that it is likely to be threatened in other areas of its range were it co-occurs with the mallard. The options for management of this situation are limited, as large areas without mallards are lacking. Captive breeding, or the management of grey duck populations on isolated islands appear the only feasible options. It seems unlikely that hybridisation can be reversed on the mainland, and a homogenous hybrid population is likely to eventually occupy the entire range of the grey duck across New Zealand.

Chapter 1

Introduction

Hybridisation in animals: the historical view

Animal hybridisation was long viewed as a rare and unimportant process, a result of incomplete species isolation detrimental to the individuals involved, and of little evolutionary consequence [Mayr 1970, Soule 1986]. Mayr [1970] defined hybridisation as: "the crossing of individuals belonging to two unlike natural populations that have secondarily come into contact." Note the stress on secondary contact in Mayr's definition. The possibility of populations hybridising that have always been in contact is not considered. Furthermore, under the biological species concept, by definition widespread interspecific hybridisation is not possible – if populations are fully interfertile, they are not 'good' species. Thus, Mayr [1970] wrote: "Not all isolating mechanisms are perfect all the time. Occasionally they fail and permit the crossing of individuals that differ from each other genetically and taxonomically. Such interbreeding is called *hybridization*. This term is difficult to define precisely and has therefore been applied to very different phenomena." He added later, referring to the fitness of hybrids, 'ecological and ethological inferiority reduces their chances of leaving offspring' and, 'backcross individuals are likely to be even more strongly inferior, owing to various imbalances of their gene complexes' [Mayr 1970]. Not least because of the use of the biological species concept and its implications, hybridisation in animals has long been viewed as a nonexistent issue, an abnormality of no consequence and little concern [Mayr 1970, Soule 1986].

Hybridisation in animals: a paradigm shift

However, Mayr's [1970] view is now regarded as unsatisfactory in many cases [Mallet 2007]. Some species that look and behave differently, and do not normally mate, can be induced to do so in captivity or by bringing them into contact that would not normally occur. For example, swordtails (*Xiphophorus hellerii*) and platyfish (*X. maculatus*), hybridise readily in captivity and are fully interfertile, but do not hybridise in nature where they co-occur widely [Hubbs 1955]. Many species of finches, and most pheasant and duck species can also be hybridised easily in captivity, and in many cases with the production of fertile offspring, but only rarely do so in nature [Schiltzhuizen 2001]. In other cases of hybridisation, almost all offspring are inviable or infertile, but there might be occasional exceptions [see for example *Chrysochus* beetles, Peterson 2005; or meadowlarks, Lanyon 1979]. Nonetheless, even these very rare exceptions may be potentially of immense evolutionary importance. Just one or two fertile hybrids might be enough to transfer an advantageous gene from one population to another, as appears to have occurred in a transfer of pesticide resistance between different crossing types of the mosquito *Culex pipiens* [Schiltzhuizen 2001]. Because of these problems and others, biologists increasingly define species as genotypic clusters rather than reproductively isolated populations [Mallet 2007].

Hybridisation as a natural evolutionary process

In contrast to the view in animals, hybridisation has been widely accepted to be a normal evolutionary process in plants since the mid-20th century [Baack and Rieseberg 2007], where hybrid zones can be hotspots of diversity and evolution, with increased

insect diversity as well as increased diversity of plants [Whitham et al. 1991]. After initial debate, it was likewise accepted to be a frequent process in many fishes [Hubbs 1955, Scribner et al. 2000]. With molecular methods now uncovering evidence of ancient, as well as recent and ongoing hybridisation in a multitude of animals, hybridisation is now also being increasingly recognised as a normal evolutionary process in all other animal groups [Arnold 1997, Dowling and Secor 1997, Arnold et al. 2001, Levin 2002].

It is estimated that about 10% of all extant animal species engage in interspecific hybridisation in the wild [Mallet 2005, Mallet 2007]. For example, bird species can remain interfertile for millions of years after populations separate (and thus appear to speciate), yet half of the crosses between species within a genus are typically fertile, with the isolation of species relying on pre-mating mechanisms [Price and Bouvier 2002]. About 10% of bird species are known to hybridise at least occasionally in nature [Grant and Grant 1992], but rates vary greatly between groups of birds [Price and Bouvier 2002]. Similarly, about 12% of European butterfly species hybridise [Schiltzhuizen 2001], as do 6% of European mammals [Price and Bouvier 2002]. Many examples from all major phylogenetic groups exist, from corals [Willis et al. 2006, Combosch et al. 2008] to primates [Cortez-Ortiz et al. 2007, Aguiar et al. 2008, Tung et al. 2008] and manatees [Vianna et al. 2006], even if rates have not been estimated.

Although hybridisation now appears to be more widespread and common than previously thought, most species do not hybridise and this is due to a variety of mechanisms that prevent successful interspecific matings. These mechanisms collectively function to create reproductive isolation between species. Reproductive isolation is often reached by other means than a simple genetic incompatibility. For example, *Drosophila*

sechellia and *D. simulans* are separated by host fruit preference only [R'Khan et al. 1991]. It is also thought that secondary sexual signals such as song in warblers [Brambilla et al. 2008] and male plumage as in ducks, pheasants and birds of paradise are common mechanisms to prevent hybridisation in the wild [Schiltzhuizen 2001].

Although hybridisation is widespread across species, rates of hybridisation within a species pair are typically below 0.1% [Price and Bouvier 2002, Mallet 2007]. Such rare hybrids have been described as 'hopeful monsters' [Mallet 2007]. Such 'monsters' are genetically exceptional individuals that may have a slim chance of making an evolutionary contribution, but if they do, it might be a large one. To think that because hybrids are rare, they are of no evolutionary consequence is a mistake. As the example of acquisition of pesticide resistance by a mosquito quoted above shows, one fertile hybrid that succeeds in backcrossing into a parental population might be all it takes for the introgression of a gene from one species to another. For the same reason, it is not the average fitness of hybrids that is of interest, but the maximum fitness [Arnold 1997, Arnold and Emms 1998, Arnold et al. 1999, Abbott et al. 2003].

The wide range of consequences of hybridisation

The consequences of hybridisation are extremely varied, and their outcome is generally unpredictable in each case. Adaptations can be gained or biodiversity reduced, and the size of the effect can range from negligible to profound. At one extreme, hybridisation can lead to extinction, or the formation of hybrid swarms, at the other it can rescue inbred populations and lead to hybrid speciation [Seehausen et al. 2008].

This unpredictability is due to the fact that the admixture of two different genomes via hybridisation results in novel, previously untested gene combinations, with resulting epistatic interactions. Such novel combinations might disrupt fine-tuned gene complexes, lead to inappropriate combinations of adaptations or imbalances in the metabolism, which in turn can lead to fitness reductions that are referred to as outbreeding depression. Alternatively, hybrids of the first generation can also experience increased vigour, referred to as heterosis [Lynch 1991]. This phenomenon is still poorly understood after decades of study, but appears to be due to some combination of dominance complementation, overdominance, and/or epistasis [Lippman and Zamir 2006], and exact causes might well differ between different species pairs. Later generations of hybrids might combine adaptations from different lineages, or show extreme characters due to additive genetic factors, thus giving them an overall advantage, or in some cases, the intermediacy of their adaptations lets them thrive in intermediate environments [Rolán-Alvarez et al. 1997]. The result does not have to be intermediate to that of the parental species, however, in some cases there are gains of adaptations that none of the parental species showed, like the increased cold tolerance of an invasive algae with hybrid origin (*Caulerpa racemosa*) that can colonise areas out of the reach of either parental species [Durand et al. 2002]. This might happen if, for example, novel patterns of gene expression arise because of regulatory incompatibilities [Landry et al. 2007]. Such hybrids might do well in extreme, not intermediate, environments [Burke and Arnold 2001].

It is therefore not unusual for first generation hybrids to appear fitter than parental species, but for subsequent hybrid generations to suffer from hybrid breakdown [e.g.

Marr et al. 2002]. The important thing to note, however, is that while variance is typically very low in first generation hybrids it tends to be extremely high in subsequent generations [Edmands 1999]. In such highly variable populations, some individuals that are fit enough to reproduce are to be expected. This can lead to introgression, or to the establishment of a hybrid population that will adapt rapidly at first due to the excess amount of variation for evolution to act on [Edmands et al. 2004].

Exogenous and endogenous selection

Both exogenous and endogenous selection have been shown to be of importance in the outcome of hybridisation. For example, hybrids between the mussels *Mytilus edulis* and *M. galloprovincialis* appear to suffer mainly exogenous selection, where phenotypes are selected irrespective of genotype [Wilhelm and Hilbish 1998]. In the chickadees *Poecilie atricapilla* and *P. carolinensis*, endogenous selection appears to play a larger role, where hybrid chicks have lower survival even in benign environments [Bronson et al. 2003]. It can be assumed that in most cases, both endogenous and exogenous selection are going to act on the highly variable hybrids of subsequent generations, though to differing degrees depending on the species pair and the individual hybrid in question.

Two studies suggest that endogenous selection can act swiftly towards an integrated genome. In sunflowers, third generation hybrids are already genetically similar to fourth to seventh generation hybrids [Rieseberg and Linder 1999]. Similarly, in copepods, after only a year of breeding after initial hybridisation (but up to 14 generations), foreign genes had been reduced from 25% to only 1.5% of the genome, no matter against which cytoplasmic background [Edmands et al. 2004]. However, in both studies, the

experimental populations were started with hybrids of equal hybrid background (i.e., all the copepods were first generation backcrosses). A more heterogeneous population may develop different dynamics. For example, since not all individuals have the same dominant genome, directional selection for one genome may be impossible or a lot slower.

Exogenous selection may be more difficult to detect, since it may concern much smaller parts of the genome. However, it is known that genes conferring adaptive advantages can selectively introgress in hybrid zones [Martinsen et al. 2001, Stein and Uy 2006], a process also possible in other hybrid populations.

Introgression: a matter of direction and amount

Any introgression that does occur through hybridisation can be large-scale, or very limited, and it might be either selective or random. The best studied examples of introgression are cases of transfer of mitochondrial DNA (mtDNA), due to relative ease of study. Examples of mtDNA capture are common, and often transfer is unidirectional: in 50 cases of more than 80 investigated hybridising species pairs only one type of mtDNA was found in hybrids [Wirtz 1999]. This might be because of mate choice, or because reciprocal crosses between species are often not equally fit. As a result, the maternal parent with faster mtDNA evolution generally has less hybrid offspring, showing that selection for mtDNA background plays a role [Bolnick et al. 2008]. The consequence is that even in repeated hybridisation events, mtDNA often migrates unidirectionally, as in hybridisations between the fish *Etheostoma caerulum* and *E. uniporum*, where mtDNA travels from the former into the latter [Ray et al. 2008], or in

frogs, where a third of *Rana ridibunda* carry mtDNA of *R. lessonae*, but none vice versa. This transfer seems to be mediated by the hybrid species between the two, *R. esculenta*, where 90% of hybrid populations carry *R. lessonae* mtDNA [Plötner et al. 2008].

Consequences at population level

The consequences of introgression due to hybridisation at the population level are likewise manifold. Most obviously, hybridisation can lead to a loss of diversity and adaptations. Recent studies on the collapse of incipient stickleback species in North America are a good example [(*Gasterosteus aculeatus* complex); Gow et al. 2006, Taylor et al. 2006]. Many incipient species have probably fused with each other in the past, a process that typically leaves no trace either in the fossil record or the genome. However, recently diverged sister species often exchange genes without loosing their species character. A study on cave salamanders and their relationship to surface-dwelling salamanders suggests that continuous and recurrent gene exchange does not prevent speciation, and might even be common during speciation events [Niemiller et al. 2008, Nosil et al. 2008]. In fact, it has been hypothesised that hybridisation may be especially common in adaptive radiations, and be a normal part of rapid speciation [Grant et al. 2005]. Introgression in rapidly diversifying taxa has indeed been shown in fish [Sousa-Santos et al. 2007], salamanders [Wiens et al. 2005], *Heliconius* butterflies [Mallet et al. 2007] and Darwin's finches [Grant et al. 2005].

Transfer of adaptations

When hybridisation leads to introgression, it can also act to increase genetic diversity within a parent species. The introgression of new genetic material can mediate more rapid introduction of novel adaptations or gene combinations than mutation could supply. A particularly well studied example of evolution by hybridisation is the rapid adaptation to drought conditions on the Galapagos in Darwin's finches by hybridisation [Grant et al. 2004]. While in years with high levels of rainfall, finch species with specialised beaks do well, in years of drought hybrids and hybrid offspring, which can utilise seeds of a wider range of sizes, have increased survival over that of the pure parent species, and their proportion in the population increases.

Another example of how introgression may lead to rapid adaptation occurs in cichlids of several genera that can acquire new colour patterns by hybridisation [Smith and Kornfield 2002, Smith et al. 2003, Streelman et al. 2004]. In this group, in which male colouration and female preference for that colouration are crucial to speciation, the acquisition of novel colour patters through introgressive hybridisation is potentially of high importance in adaptive radiations. Green algae, plant, insect, fish and reptile species have all been shown to gain adaptations necessary to invade new habitats in this way [Lewontin and Birch 1966, Huxel 1999, Durand et al. 2002, Blumler 2003, Kolbe et al. 2004, Nolte et al. 2005, Kolbe et al. 2007].

These examples support the view that, far from always being maladaptive, hybridisation may sometimes be the key to increased fitness. A successful 'hopeful monster' may be disproportionately successful, and may even found an entire new lineage or species. Not surprisingly, advantageous traits can introgress very rapidly. In

cottonwood (*Populus fremontii x P. angustifolia*) hybrids, some loci introgress rapidly over a hybrid zone, while others seem to drift neutrally or be selected against, which suggest that the quickly introgressing alleles might be advantageous [Martinsen et al. 2001]. In animals, the yellow colouration of golden-collared manakins (*Manacus vitellinus*) has introgressed rapidly into white collared manakin populations (*M. candei*), as it is sexually selected, and males with yellow colouration are preferred by females [Stein and Uy 2006].

Non-genetic aspects: overlooked and understudied

Sometimes payoffs might be of a non-genetic nature. Interestingly, collared flycatcher (*Ficedula albicollis*) females appear to gain better territories when mating with pied flycatcher males (*F. hypoleuca*), rather than with their own species [Wiley et al. 2007]. This may enable them to lay more eggs and raise more chicks, even if some of those chicks might have lowered fitness. Although extrapair copulations could still ensure that at least some of the young are not hybrids [Wiley et al. 2007], the direct non-genetic benefits of hybridisation to an individual have been poorly studied.

Hybridisation as a facultative, adaptive response

Under some circumstances, hybridisation has been shown to be one of a set of adaptive reproductive strategies. Female collared flycatchers (*F. albicollis*) that pair extraspecifically with pied flycatchers (*F. hypoleuca*) reduce the cost by siring a large number of offspring by engaging in extrapair copulations with conspecific males, and by biasing the offspring ratio towards males. The latter is an advantage as female hybrids are

sterile, while males are not [Veen et al. 2001]. Under some circumstances, this can be an optimal strategy for the female, though not for the male [Veen et al. 2001].

In some cases, hybridisation may be facultative depending on current conditions. For example, female spadefoot toads (*Spea bombifrons*) hybridise with the more rapidly developing *S. multiplicata* only when water levels are low, which means that the temporary pools that their tadpoles inhabit will dry up soon [Pfennig 2007]. Hybrids grow up faster than non-hybrid offspring, and survival rates are therefore higher for hybrids when water levels are low. Females can increase their fitness by hybridising only in those conditions when this is the optimal strategy [Pfennig 2007]. More studies investigating the possibility of hybridisation as a facultative strategy are clearly needed.

Hybrid speciation

Though debated for a long time, evidence that hybridisation can also lead to the evolution of new animal species is becoming overwhelming. This seems to be an especially common process among fishes. One example is the African cichlids. *Neolamprologus marunguensis* is a cichlid species that arose by introgression, showing a mosaic of mtDNA haplotypes and microsatellite loci from both ancestors [Salzburger et al. 2002]. A well-known example of an amphibian species that is produced by hybridisation is the frog *Rana esculenta*, which is derived from crosses between *R. lessonae* and *R. ridibunda* [Abt and Reyer 1993]. Candidate cases of speciation involving hybridisation of mammals are rare, but hybrid origins have been suggested for the wisent (*Bison bonasus*) [Verkaar et al. 2004] and the hamadryas baboon (*Papio hamadryas*) [Wildman et al. 2003].

In one case, speciation through hybridisation has occurred within a human time frame. The tephritid fruit flies *Rhagoletis mendax* and *R. zephyria*, which usually live on different host plants, started hybridising when an introduced plant (*Lonicera* spp.), a hybrid itself, proved a suitable host to both. In the 250 years since the introduction of the host plant, the population on the *Lonicera* plants has developed its own lineage, with no first generation hybrids being detected. The hybrid population has been named as *R. pomonella*, indicating species status [Schwarz et al. 2005, Schwarz et al. 2007].

Hybridisation as a conservation issue

While hybridisation is a common and a natural process, with an important role in evolution, it is also sensitive to human disturbance. Following human actions, hybridisation rates can both increase or decrease, and hybridisation can be induced or stopped. The problem of increased hybridisation is the more imminent and more obvious of the two, at least from a conservation perspective, and has thus received the most attention. It is unknown how much hybridisation is caused by human interference, but it has been estimated that about 50% of recorded cases of fish hybridisations are due to human influences like agriculture, introductions and habitat alterations [Scribner et al. 2000].

Due to human influence, geographic and ecological barriers are breaking down world wide, bringing previously isolated species into contact and homogenising environments [Chapin et al. 2000]. Such human actions include voluntary introductions and accidental transport, but also habitat alterations that result in range expansions of species, or increased contact between allopatric species. This homogenisation of the environment

may lead to a widespread reversal of speciation [Rhymer and Simberloff 1996, Seehausen 2006, Seehausen et al. 2008]. The ensuing hybridisation can then lead to the erosion of genetic diversity, and may lead to loss of adaptation, to extinction, and to the loss of evolutionary potential [Woodruff 2001, Rosenzweig 2001, Myers and Knoll 2001]. It thus not only threatens species alive today, but may constrain the evolutionary potential of such species in the future.

The effect of hybridisation on evolutionary potential is poorly studied but it could be critical in managing the conservation of threatened species. Evolutionary potential can be defined as the ability of a population to successfully adapt to changes in the environment, present or future [Frankham et al. 2002, Frankham 2005]. It is not identical to heterozygosity or genetic diversity *per se* [Allendorf 1986]. For example, a population of lower diversity that contains the most relevant genes for crucial adaptations may have a higher evolutionary potential than a highly diverse population lacking such variants. Evolutionary potential is difficult to determine in any given population, partly because it is difficult to predict future circumstances and therefore which genetic variants will be of importance. Thus, hybridisation has the potential to either increase or decrease evolutionary potential; it may even decrease for a species globally while increasing for a population of that species locally. An increase can happen for example if a population aquires a crucial adaptation via hybridisation, while a decrease may occur if a single mixed population looses local adaptations of the two previous populations or breaks up co-adapted gene complexes. In a conservation context, decreased evolutionary potential through hybridsation is likely to reduce the long-term viability of an endangered population. On the other hand, hybridisation that increases evolutionary potential can still

be controversial for species conservation, particularly if it occurs in a situation where hybridising species would not be in contact without human influence.

The diverse range of issues with hybrids in conservation

Human induced hybridisation poses many conservation problems [Rhymer and Simberloff 1996, Haig 1998, Mooney and Cleland 2001, Levin 2002]. Introduced species hybridising with native ones and thus threatening the integrity, diversity or existence of native populations pose one of the largest problems, both in plants [Abbott et al. 2003] and animals, where examples include such diverse groups as crayfish [(*Orconectus*); Roush 1997, Perry et al. 2000], fish [Scribner et al. 2000, Perry et al. 2002], newts [(*Ambystoma*); Riley et al. 2003], partridges [(*Alectoris*); Barbanera et al. 2005, Tejedor et al. 2007], hares [(*Lepus*); Andersson et al. 1999] and deer [(*Cervus*); Lowe and Gardiner 1975, Abernethy 1994, Goodman et al. 1999]. In the case of partridges, captive bred hybrids are released into the wild to bolster hunting stock, thus altering the genetic integrity of the wild stock [Barilani et al. 2007]. Hybridisation is also a problem in captive breeding programs, where subspecies or species may have access to each other that would not do so in the wild, as in the case of the Saudi gazelle (*Gazella saudiya*) and the Chinkara (*Gazella bennetti*) [Rebholz and Harley 1997], an occurance certainly undesirable in most cases of breeding for conservation. In many of these cases, the heightened invasiveness of introduced organisms following hybridisation with natives is a major concern.

Habitat alterations can bring species into contact with congeners when they expand ranges. For example, the spotted owl (*Strix occidentalis*) began to hybridise with the

previously allopatric barred owl (*S. varia*) after deforestation of redwood forests [Hamer et al. 1994], further endangering the already threatened spotted owl. Habitat alteration may threaten species integrity even without range expansions. Increasing turbidity due to runoff laden with fertiliser and sediment in Lake Victoria and Lake Malawi is leading to increased hybridisation between cichlid species, since many species are interfertile and separated by mate choice recognition based on colour patterns [Seehausen et al. 1997, Streelman et al. 2004], which are harder to distinguish in turbid waters. Human actions have been suspected to cause a number of hybridisations within primates [Detwiler et al. 2005], and the widespread hunting of fur seals (*Arctocephalus*), leaving some populations very small, appears to have fuelled hybridisation between some species during re-colonisations, possibly because the availability of conspecifics is low [Lancaster et al. 2006, Kingston and Gwilliam 2007, Lancaster et al. 2007]. In Darwin's finches (*Geospiza* spp.), the role of human influence has been shown in more detail. In areas where human densities are low, two beak sizes in two species persist, but where humans are dominant, a medium-sized beak, mediated by hybridisation between the species, is most common [Hendry et al. 2006]. In this latter case, while hybridisation is clearly mediated by human influence and may slow or even reverse ongoing speciation, hybridisation could well be adaptive to a landscape altered by humans. Whether this is desirable or not becomes an ethical argument difficult to solve.

Hybrid swarms

The extreme cases of hybridisation in which hybrid swarms are formed pose special conservation concerns since once they have formed, they cannot usually be undone and it

may only be possible to conserve the genes of participating species in mixture. Several cases of hybrid swarms have received scientific attention. The Pecos pupfish (*Cyprinodon pecosensis*) and the Leon Springs pupfish (*C. bovines*) both hybridise with sheepshead minnows (*C. variegatus*) which were introduced as bait fish. Hybridisation now threatens both pupfish species' integrity [Wilde and Echelle 1992, Childs et al. 1996, Echelle and Echelle 1997]. Due to the release of trout for fishing purposes, a number of non-native species or subspecies now hybridise with native ones, often very extensively, in large areas of North America. Examples include the hybridisation of rainbow trout (*Oncorhynchus mykiss*) with cutthroat trout (*O. clarki*) [Ostenberg and Rodriguez 2002, Ostenberg and Rodriguez 2004, Ostenberg et al. 2004, Rubidge and Taylor 2005]; subspecies of the latter (*O. clarki lewisi* x *O. c. bouvieri)* [Gyllensten et al. 1985, Forbes and Allendorf 1991], and rainbow trout (*O. mykiss*) with apache trout (*O. apache*) [Dowling and Childs 1992]. The diversity of the entire complex of North American trout taxa may be in peril if releases continue.

The hybridisation of American black ducks (*Anas rubripes*) and mallards (*A. platyrhynchos*) in North America was not caused by human introduction, but by alteration of the habitat leading to a spread of the mallard into the range of the black duck [Maisonneuve et al. 2000]. The two species are measurably less genetically distinct now than they were a hundred years ago, and will probably continue interbreeding until they form one homogenous taxon [Mank et al. 2004]. Introduced crayfish and mussels hybridise with native congeners, a frequent but often overlooked problem in the conservation of freshwater species [Perry et al. 2002]. A similar problem exists between domesticated forms and closely related wild species [Randi 2008]. Examples include

wildcats (*Felis silvestris*) and domestic cats (*F. domesticus*) [Beaumont et al. 2001, Pierpaoli et al. 2003, Oliveira et al. 2008], wolves (*Canis lupus*) and dogs (*C. familiaris*) [Randi and Lucchini 2002, Ciucci et al. 2003, Vila et al. 2003, Verardi et al. 2006], and dogs and the most endangered canid species, the Ethiopian wolf (*Canis simensis*) [Gottelli et al. 1994]. Bison (*Bison bison*) are also vulnerable to introgression by cattle (*Bos taurus*) [Halbert and Derr 2006], and critically endangered markhor (*Capra falconeri*) in zoos have been shown to have hybridised with domestic goat (*C. aegagrus hircus*) [Hammer et al. 2008]. Hybridisation with domestic species is probably maladaptive and thus undesirable from a conservation viewpoint in all cases – it is unlikely that traits arising under domestication will prove adaptive in the wild.

Setting conservation guidelines

The situation of hybridisation and how to deal with it can sometimes be confusing to conservation professionals. On the one hand, for a long time, in the US and in many other nations, hybrids automatically lost status of protection, but this hotly debated 'hybrid policy' was finally relaxed due to the realisation that hybrids were a natural part of evolving systems [Allendorf et al. 2001], and that introduction of new genes via hybridisation may sometimes be a taxon's best chance for survival, as in the case of the Florida panther (*Puma concolor coryi*) [Pimm et al. 2006]. With the realisation that hybridisation may be a vital evolutionary mechanism, there has even been a call that instead of species, in some cases species complexes need to be conserved with their potential for gene flow intact [Whitham et al. 1991, de Marais et al. 1992]. It can even be argued that increased rates of hybridisation can be a response by some organisms to the

selection pressures introduced by human disturbance [Arnold et al. 2001]. As in the case of Darwin's finches, it might allow faster adaptation to the new environment and circumstances. Even though induced by human activity, allowing continued hybridisation might still be a beneficial process to the aim of conserving a species complex.

On the other hand, despite the wide range of affected species, the problem of widespread hybridisation induced by human activity is often underappreciated [Rhymer and Simberloff 1996]. Such extensive hybridisation is an awkward conservation problem. Detection and management can be extremely difficult, and there are no fast and ready solutions as management strategies have to be developed for each individual case [Allendorf et al. 2001, Edmands 2007]. In the case of advanced introgression, there might not be much that can be done.

Hybridisation in waterfowl

Waterfowl appear particularly prone to hybridisation with a rate of 40% of species being known to hybridise, rather than the 10% within birds in general [Grant and Grant 1992]. The mallard (*Anas platyrhynchos*) in particular, having been brought into contact with a number of other species by worldwide release as a game bird and by the spread of the domestic duck (whose ancestor was the mallard), is known to hybridise with at least 23 other species of dabbling ducks (*Anas* spp.), with about 20% of these crosses being fertile [Marchant and Higgins 1990].

There is considerable controversy concerning the phylogeny of the Anseriformes, including the genus *Anas*. Genetic studies are indicating many cases of ancient hybridisation that led to transfer of genes, often detected as transfer of mtDNA [see e.g.

Johnson and Sorenson 1991, Donne-Goussé et al. 2002]. Molecular methods have however also helped revealed substantial differentiation within the genus *Anas* [see e.g. Hawaiian duck and mallard; Browne et al. 1993; Meller's duck and mallard; Young and Rhymer 1998], with particularly the African and Pacific clades appearing well differentiated [Johnson and Sorenson 1991]. The ability of duck species to hybridise is commonly retained through long evolutionary time periods, and a consensus is emerging that an ability to hybridise does not negate species status [Rhymer 2006].

Why should the waterfowl in particular be so prone to hybridisation? Most duck species sharing the same range and habitat are fully fertile when crossed but such crosses are generally rare in terms of the number of individuals involved. Hybrids are seen occasionally in the wild, but not as commonly as might be presumed from the high number of species involved. Some are just common enough to be listed in the more thorough guide books. Whenever one is spotted by bird watchers, note is taken and individuals can sometimes be tracked over years, an indication perhaps of the interest generated by their rarity. Thus, hybridisation in ducks appears to involve few individuals but nonetheless is widespread across species.

The species barriers in ducks are normally kept up by pre-mating isolation. Waterfowl often have pronounced sexual dimorphism, and males are very ornate while females look similar to each other in camouflage-coloured plumage. While females have genetic clues as to what a male should look like, males gain this knowledge by imprinting on their mothers [Bauer and von Blotzheim 1968, Williams 1983, ten Cate and Vos 1999]. Nevertheless, males will still court females of other species when given the chance and especially if not many females of their own species are present, but females will

decline their advances. This system works so well that completely fertile species will remain entirely separate despite spending their lives on the same lakes or rivers. It is possible that because of the almost complete pre-mating isolation, a need to evolve post-mating isolation never existed. There is also the possibility that keeping the option of genetic exchange open might be an evolutionary advantage allowing acquisition of advantageous alleles.

Despite the maintenance of species boundaries in the wild, under certain conditions, the barriers to hybridisation can break down. This seems to be the case especially in captivity, where females cannot evade the advances of males of other species, or where there might be a lack of conspecific partners. There might also be cases of mis-imprinting, in which a male is imprinted on the females of another species, and a female does not manage to evade the attempts by the male. Waterfowl are among the few groups of birds to possess an intromittent organ, and are therefore capable of forced copulations, which are very common among some species, including the mallard [McKinney et al. 1983, Cunningham 2003]. One comparative study concluded that brood amalgamation, which is likely to lead to occasional mis-imprinting, is a better predictor of hybridisation between species than the presence of forced copulations, but both played a role [Randler 2005].

Mating isolation can also break down when two species that have been separated for an evolutionarily significant time span come into secondary contact. In such cases, it is possible that despite a significant divergence, the specific cues to allow pre-mating isolation are not perfected, since no selection pressure existed to evolve them. Females might not be capable of adequately distinguishing between males of their own and the

other species, or they might not be able to fend off forced copulations. In some cases, widespread hybridisation, and even extinction, can be the result.

The mallard and grey duck hybridisation in New Zealand

In New Zealand, extensive hybridisation occurs between the native grey duck (*Anas superciliosa superciliosa*) and the introduced mallard (*A. platyrhynchos*). The mitochondrial DNA control sequences of the two species diverge by 7-11% [Rhymer et al. 2004]. The two species also differ markedly in a number of phenotypic characters. Most strikingly, mallards are sexually dimorphic, the males sporting a bright green head, yellow bill, chestnut coloured chest and grey body with orange legs and a distinctive black tail curl feather. Female mallards are dappled brown [Marchant and Higgins 1990, Heather et al. 2000]. Grey ducks (also called black ducks in Australia and the Pacific) of both sexes are of a darker more subdued brown with grey legs and bill. The head is light cream with two striking black stripes running from the bill to the back of the head. The speculum on the wing of grey ducks is green, while that of mallards is blue with clear white edges either side. Grey ducks are also considerably smaller than mallards [Marchant and Higgins 1990, Heather et al. 2000]. Grey duck drakes guard the nest site, which tends to be in tree holes or old corvid nests [Marchant and Higgins 1990], while male mallards make no contribution whatever to nesting, which happens on the ground. On average, grey ducks lay smaller clutches and also tend to nest only once per season, not two or three times like mallards [Marchant and Higgins 1990]. Forced copulations are a lot more common among mallards, if they occur amongst grey ducks at all [Heather et al. 2000]. Differences in habitat preference or diet are also possible – it is often said that

grey ducks prefer more wooded sites than mallards [Marchant and Higgins 1990] – but difficult to substantiate. It can be assumed that because of their greater size, and therefore longer reach under the water surface, mallards can forage to greater depths than grey ducks [Haddon 1998]. Despite all these differences, hybrids became a common sight after the introduction of mallards to New Zealand.

A history of the mallard and grey duck hybridisation

Mallards were first introduced to New Zealand in the second half of the 19th century from British stock imported via Australia by the Canterbury Acclimatisation Society [Knox 1969, Heather et al. 2000]. However, these were mainly used as breeding stock, and the first serious attempts to establish the species were undertaken in 1908/1909. While a putative hybrid was shot in 1917 [Knox 1969, Marchant and Higgins 1990], the introduction was initially not very successful. The Natural History of Canterbury of 1927 does not even mention the mallard, but the native grey duck is said to be reduced by shooting [Speight et al. 1927], which may have made it easier for the mallard to invade following later liberations.

In the 1930s and 1940s introductions of mallards were attempted on a much larger scale, this time with animals of American origin [Knox 1969, Heather et al. 2000]. The mallard spread rapidly from 1950 onwards, and with it hybridisation. In the breeding season 1967/1968, it was estimated that 58% of pairs in the Waikato district were mallards, 38% were grey ducks and 4% were mixed pairs [Hitchmough et al. 1990]. By that time, both species were living side by side in most parts of New Zealand, and it had been noted that mallards tended to dominate in closely settled areas and the entire eastern

South Island, and hybrids were a widespread phenomenon [Knox 1969]. By 1977, mallards made up an estimated 82% of the population [Gillespie 1985]. In 1981/1982 hybrids made up more than half (51%) of the population, and there were only 4.5% grey ducks left [Gillespie 1985]. In addition, most hybrids appeared more mallard-like than grey-like [Gillespie 1985]. A report by the Department of Conservation, using phenotypic characters of more than 2000 ducks collected in the 1998 hunting season, showed that animals with hybrid or mallard characteristics dominated in all areas sampled (ranging from 74% on the west coast to 99% in Southland). However, by comparing genotype and phenotype of animals of known hybridisation status from a breeding program, the same study also showed that phenotypic characters were unreliable indicators of hybrid status [Williams 1998]. Therefore, in the absence of genetic data, nobody really knows how much hybridisation occurs, or the current state of the parent populations.

Prior studies aiming to understand the causes of the mallard and grey duck hybridisation

Although several studies have been conducted into different aspects of the hybridisation of the grey duck and mallard, knowledge is sparse of why the species barriers failed, and what determines the dynamics within the resulting hybrid population. It is known that mitochondria introgress bi-directionally, so initial hybridisation was not only by mallard drakes and grey duck females, as might be expected if females are attracted to or are aggressively claimed by the more colourful and larger mallard drakes [Rhymer et al. 1994]. Another study employing allozymes found only a distinct lack of polymorphisms in both species in New Zealand [Hitchmough et al. 1990]. In controlled

breeding experiments, the species differed in reproductive capacity, with hybrids intermediate to the more fecund mallards and less productive grey ducks. There was also some breeding asynchrony, but no clear differences in embryo mortality or offspring sex ratios [Williams and Roderick 1973, Haddon 1984]. Band recovery data showed also no significant survival differences between the two species [Caithness et al. 1991].

The aims of this thesis

The aim of my PhD was to shed more light on the mechanisms and processes of extensive interspecific hybridisation, using the grey duck and mallard species pair as a study system, but also using other systems where appropriate. In particular, a better understanding of the causes and consequences of hybridisation was seen as a necessary step in the development and establishment of guidelines for the management of hybridisation in conservation.

My first objective was to accurately assess the current situation of grey duck and mallard on the West Coast of the South Island by means of a survey (Chapter 2), as no comprehensive data were available on the current state of hybridisation between the two species in the area thought to support the largest remaining number of grey ducks. These data were also used to investigate any spatial differences within the study areas in the South Island (Chapter 2). Additionally, the hypothesis that grey ducks are more numerous in more natural habitats was tested, so as to allow inferences about possible persistence of grey ducks in less disturbed habitats (Chapter 2).

The second aim of my thesis was to investigate mating patterns of ducks in the wild (Chapter 3), as mating patterns in widely hybridising populations provide a key to

understanding the dynamics of hybridisation, for understanding its history and predicting its future. Data were collected on the phenotypes of partners in pairs, and used to assess if mating was assortative within parental species, or random. Of particular interest was the behaviour of hybrids themselves, an issue often understudied and poorly understood.

To further assess the situation of the grey duck and mallard hybridisation, a genetic hybrid test was developed using genotyping of microsatellite loci (Chapter 4). To this aim, samples from ducks of both parental species were genotyped, and so were samples from New Zealand putative hybrids. This test was designed, in particular, to show how accurate or inaccurate the assessment of hybrid status by phenotype is for an individual.

The relative fitness of hybrids is another important factor to understand and predict dynamics in the hybridising population. Thus I selected several health-related measures, such as parasites and immunoresponse, to determine if mallard-like ducks appeared healthier, according to these measures, than grey-like ducks (Chapter 5). To this aim, live ducks were captured and shot ducks collected and tested for a range of health-related measures, to measure any potential differences between parental species and their hybrids.

Next, a mathematical model was used to test the completeness of the understanding of the process of hybridisation, and help predict the future of the grey duck and mallard, both in New Zealand and elsewhere (Chapter 6). The model was programmed to simulate the development of a hybrid swarm, and the results of all previous chapters were combined with data from the literature for best estimates of parameters. The model was tested using two different cases of hybridisation, one of them the mallard and grey duck case, expecting that it would correctly predict different outcomes. It was then used for

predictions, within the limits possible to a model, of the future of the New Zealand or any other sympatric populations of grey duck and mallard.

In the last part of my thesis I switched from duck hybridisation and conducted an experimental study instead with hybridisation between fruit flies. I chose fruit flies as a model system as this allowed me to address a basic question of hybridisation that was not possible to test with ducks. The hypothesis was that an individual faced with the choice between a closely related individual and that of another species would be more likely to hybridise than if a non-related conspecific had been available, and thus that levels of inbreeding can influence hybridisation rates. *Drosophila* mate choice trials were used for this study (Chapter 7). Male and female mate choice preference was assessed when giving each individual a choice between partners of their own species and those of another species.

The structure of this thesis

Due to the diverse matter of this thesis, the main body of the thesis is presented as a series of chapters, each of which was designed to stand alone to a large degree. While necessitating some repetitions, especially in the introductions and methods sections of different chapters, it enables the reader interested in only one or two of the aspects of this thesis to study the sections of interest without having to read the entire thesis.

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Chapter 2

The last stand: a survey of mallard, grey duck and their hybrids along the West Coast of the South Island of New Zealand

Abstract

A survey of the introduced mallard (*Anas platyrhynchos*), native grey duck (*A. superciliosa*) and their hybrids was conducted along the West Coast of the South Island, and near the cities of Nelson and Christchurch. Phenotype was used to identify hybrids and parental species. No populations of ducks with a pure grey duck phenotype were found at either Nelson or Christchurch, and only 10% of the total population on the West Coast were classified as pure grey duck phenotype. Hybrids typically made up about 50% of each population. These numbers reflect phenotype only, and it is possible that genetic introgression between the two species is higher. At the moment, the only large populations of relatively pure grey ducks were found south of 43° 15' S. I suggest this is due to the Southern Alps delaying colonisation of mallards of the west coast by 20-30 years. My survey indicates grey ducks have declined compared to historical data. If current trends continue, populations of phenotypically pure grey ducks may be extinct on the South Island in 10 or 20 years.

Introduction

Interspecific hybridisation is gaining recognition as a serious conservation problem, potentially leading to the extinction of species via the loss of genetic integrity [Rhymer and Simberloff 1996, Mooney and Cleland 2001, Allendorf et al. 2001, Levin 2002, Perry et al. 2002]. Humans can induce or increase hybridisation through habitat changes, or the introduction of previously geographically isolated species [Hendry 2006, Seehausen 2006, Seehausen et al. 2008].

Waterfowl (Anseriformes) appear to be particularly prone to hybridisation. While interspecific hybridisation has been recorded in about 10% of bird species, the rate is more than 40% in waterfowl [Grant and Grant 1992], and about 20% of the hybrid crosses within the dabbling ducks (genus *Anas*) produce fertile offspring [Marchant and Higgins 1990]. For example, the mallard (*Anas platyrhynchos*) is known to hybridise with 23 other species of dabbling ducks [Marchant and Higgins 1990, del Hoyo 1992]. In most cases of interspecific hybridisation the integrity between the two species stays intact, with at most some introgression taking place [e.g. Peters et al. 2007]. However, in New Zealand, the introduced mallard hybridises widely with the native grey duck (*Anas superciliosa*), threatening the integrity of the latter species [Drilling et al. 2002].

Mallards were first imported into New Zealand about 1870 by the Canterbury Acclimatisation Society for the purpose of establishing them as a game species in the Canterbury area [Knox 1969, Heather et al. 2000]. The numbers released were initially low and the species failed to establish; a natural history of Canterbury in the 1920s does not even mention the mallard [Stead 1927]. Nonetheless, a putative mallard/grey duck hybrid was shot in 1917 [Knox 1969, Marchant and Higgins 1990], and renewed

introduction efforts from 1930 to 1960 firmly established the mallard. In the first few decades after their introduction, mallards made up only ~5% of dabbling ducks nationally [Knox 1969, Marchant and Higgins 1990, Heather et al 2000], but by ~1970, mallards were widespread and the most prevalent duck in the eastern South Island [Gillespie 1985, Grant and Gillespie 1985]. Surveys in 1985 estimated that grey ducks had declined to only 20% of ducks nationally, and that 25% of all ducks were hybrids – with 50% hybrids and less than 5% grey ducks in some regions [Grant and Gillespie 1985, Marchant and Higgins 1990]. By the end of the 20th century, the number of mallards rose to 3 - 5 million nationwide [Heather et al. 2000], while the number of grey ducks fell from an estimated 1.5 million in 1970 to less than half a million in the 1990s [Marchant and Higgins 1990, Heather et al. 2000]. It is thought that all populations now contain hybrids [Marchant and Higgins 1990]. While none of the studies above may by itself be conclusive evidence for a sustained spread of hybridisation over the entire country, or of increased hybridisation over time, taken together they suggest that the process of hybridisation between mallard and grey duck has not reached an equilibrium and grey ducks may still be declining.

The most comprehensive and recent data on the status of the grey duck were collected by surveying hunters from 1970 to 1990 [Williams 1998]. Hunters reported the proportion of grey ducks to be 50% or more at 7 of 12 survey regions in 1970, but by 1990 only the West Coast region reached that figure while other areas reported as few as 1% grey ducks. At the same time, the proportion of hybrids was estimated to have increased to 41% nationally [Williams 1998]. However, identification of hybrids by hunters may not always be reliable [see e.g. Thulin et al. 2006]. For example, when

hunters were asked to categorise ducks in areas where mallards were prevalent, any duck with a similarity to a grey duck was often perceived as a pure grey duck even if signs of hybridisation were clear, while in areas where grey ducks were still common, clear hybrids were often perceived to be mallards [pers. obs.]. While hunter surveys are helpful to assess temporal and geographical differences in relative grey duck numbers, they may well give a biased estimate of the state of pure grey ducks, and offer little information about the hybridisation between the species.

In this study, I assess the current status of the grey duck on the South Island of New Zealand to (1) document the advance of hybridisation since the last surveys, and (2) investigate spatial patterns in hybridisation. I concentrated my study along the West Coast of the South Island as this is where previous surveys had found the largest numbers of grey ducks [Williams 1998], and where I thus expected to find more individuals of a grey duck phenotype than in the eastern Canterbury area. I then compared these results with historical data from the literature. My surveys were also used to investigate if grey ducks occur in less modified habitat than mallards, and thus whether the persistence of grey ducks on the West Coast may be a function of lower levels of habitat disturbance in this region.

Methods

Two surveys were conducted, one in late June 2006 and the other in early July 2007. On both occasions the weather was cold, frosty and sunny, offering excellent visibility. Binoculars were used where necessary.

Survey locations

I surveyed the relative abundance of mallards and grey ducks at eight locations. These were selected to span a north-south trajectory along the West Coast of New Zealand, from Nelson to Haast, at approximately equally distant intervals (Figure 1). The locations were: Nelson (41˚ 17’ S, 173˚ 15’ E), Murchison (41˚ 47’ S, 172˚ 19’ E), Westport (41˚ 45’ S, 171˚ 37’ E), Greymouth (42˚ 27’ S, 171˚ 12’ E), Hokitika (42˚ 44’ S, 170˚ 58’ E), HariHari (43˚ 8’ S, 170˚ 34’ E), the Glaciers (both Franz Josef, 43˚ 21’ S, 170˚ 10’ E, and Fox, 43˚ 28’ S, 169˚ 59’ E, areas combined), and Haast (43˚ 52’ S, 169˚ 2’ E). Christchurch (43˚ 32’ S, 172˚ 37’ E) was used to sample the current structure of a population on the east coast of the South Island. Because of very low numbers of ducks recorded in Murchison (N=18), this location was excluded from the analysis and not surveyed in the second year.

Each location was surveyed for one day, beginning shortly after sunrise until late afternoon (approximately 9 hours) or until all bodies of water had been surveyed. I searched all suitable locations such as lakes, rivers, ponds and sewage treatment works in an area of approximately 5-10 km around each of the 8 locations. Sites to search were identified beforehand using maps and satellite pictures. Each site was searched systematically and notes taken of all ducks seen. Most flocks encountered were of a small size (up to 15 individuals), making it feasible to survey all birds encountered in these flocks. In some locations, particularly central Christchurch, flocks were commonly of a larger size. If the ducks were sitting on land, it was usually still possible to record each duck in turn. In the rare cases where this was impossible, for example where ducks started moving during observation in response to being fed by humans, observation of

that flock was stopped to prevent registering any individuals twice, and I moved on to the next flock.

Species and hybrid categories

All ducks observed were classified as belonging into one of the following five phenotypic categories: (1) pure mallard, (2) mallard-like hybrid, (3) intermediate hybrid, (4) grey-like hybrid, or (5) pure grey duck. I used a previously developed plumage score [Rhymer et al. 1994; see appendix] as a guideline to classify birds into each category. The plumage score could not be used exactly as detailed in Rhymer et al. [1994] as some diagnostic body parts (e.g. legs or speculum) were not always visible. In these cases, I used all diagnostic features visible, ignoring those that I could not evaluate. If an individual's head or bill were tucked under a wing, I would walk up close enough for the duck to lift its head and thus make them visible. The same strategy was employed to make ducks get up if they were sitting on land with their legs invisible underneath them. To be classified as either a pure mallard or a pure grey duck, an individual could not display any signs of admixture with the other species. Ducks with some signs of hybridisation, such as orange feet in an otherwise grey duck phenotype, or lack of green on the head in a male mallard, were classified in the grey-like or mallard-like categories, respectively. Intermediate hybrids were those birds with a number of intermediate traits between the two species, such that they could be assigned to either species about equally.

The sex of each bird was recorded when possible. The sex of mallards is easily determined by plumage colouration and features such as the curled tail feathers of drakes. Most hybrids could also be sexed on the parts of their phenotype that is characteristic of

male mallards. However, since grey ducks are monomorphic, all grey ducks and some grey-like individuals could not be assigned a sex. In some cases, behaviour in courting allowed a clear assignment. The remaining cases were marked as unknown sex.

Historical data

To determine whether the proportions of the two species, and their hybrids, have changed over time, I compared my survey data with that published by Williams (1998). This report gives estimates of the proportions of grey ducks and mallards shot in 1970 and 1990 in each region of New Zealand. Unlike my survey, there was no hybrid category in these earlier surveys, so hunters classified intermediate ducks as one of either parent species. Thus, for comparative purposes I modified my data by leaving out the intermediate hybrid category, and combining the mallard-like and pure mallard categories, and the grey-like and pure grey duck categories.

Apart from these differences in reporting method and sampling method, there was also a difference in the area sampled. Most of my data was collected in farmland and towns, while the data collected by hunters was from farmland and lakes. This difference should not affect the outcome of my comparison unless the relative numbers of each species vary dramatically with habitat type. However, it is likely that my comparison of duck types in central Christchurch may not be representative of the eastern South Island region as a whole as the only historical data available was from rural parts of north Canterbury.

Habitat segregation

It has been suggested that grey ducks prefer less disturbed, more natural habitats, while mallards prefer human dominated habitats [e.g. Heather et al. 2000]. To test for habitat preferences, the plumage types of ducks seen in Haast and the glacier areas in natural habitats (rivers, lakes, estuaries), were compared to those seen in human dominated ones (farmland, sewage ponds). Only the data from the Haast and glacier areas were used as only in these areas were there both sufficient ducks of all five plumage categories, and sufficient observations on both habitat types, to allow for a meaningful test.

Statistical methods

Statistical tests were performed using Excel spreadsheets and Minitab (version 15.1.0.0). Because counts should follow the Poisson distribution, χ^2-tests were used for count data. Expected values for comparison between years, sexes, and locations were calculated in standard χ^2-test method, using the formula: (total number of ducks in plumage category)*(total number of ducks in year or sex or location) / (grand total of ducks). This method tests if the populations observed differ from a population that would ensue if all plumage types appeared with equal likelihood over years, sexes or locations.

Results

A total of 3530 individual ducks were included in the survey. This includes 1610 ducks observed in 2006, and 1920 ducks in 2007.

Differences between the two survey years

Significant differences were found between the data collected in the two years ($\chi^2 = 30.8$, df = 4, $p < 0.001$). Accordingly, analyses were performed for each year separately as well as the total set.

Differences between the sexes

The number of female and male ducks varied significantly between categories when data for both years were combined ($\chi^2 = 44.6$, df= 4, $p<0.001$). For example, there were more male than female mallards, and more female than male mallard-like hybrids. There was also a slight surplus of grey duck females, but since grey ducks cannot always be reliably sexed, this result could be an artefact. There also appears to be a general surplus of males in the population, with 28.4% more males than females (Table 1).

Table 1. Numbers of ducks in each hybrid category and sex, with row and column totals.

	mallard	**mallard-like hybrid**	**intermediate hybrid**	**grey-like hybrid**	**grey duck**	**total**
male	877	524	316	128	57	1902
female	530	529	260	96	66	1481
unknown	0	0	0	71	76	147
total	1407	1053	576	295	199	3530

Differences between locations

Overall, 54.5% of ducks seen were identified as hybrids, and only 5.6% as pure grey duck. Even considering the West Coast only, 55.7% of ducks were classified as hybrids, although the proportion of pure grey ducks was somewhat higher (10.7% of 1841 ducks surveyed).

The relative distribution of grey ducks and mallards along the West Coast appears to follow a gradient, from more pure mallards and mallard-like hybrids in the north to more pure grey ducks in the south, with the area around Haast and the Glaciers the only places where pure grey ducks comprise more than 25% of the population (Figure 1). The proportions of mallards and grey ducks differed significantly between locations (χ^2= 789.4, df = 28, $p < 0.001$). In contrast, only 0.17% of 1197 birds observed in Christchurch were pure grey ducks by phenotype, while in Nelson not a single grey duck was seen (out of 492 birds).

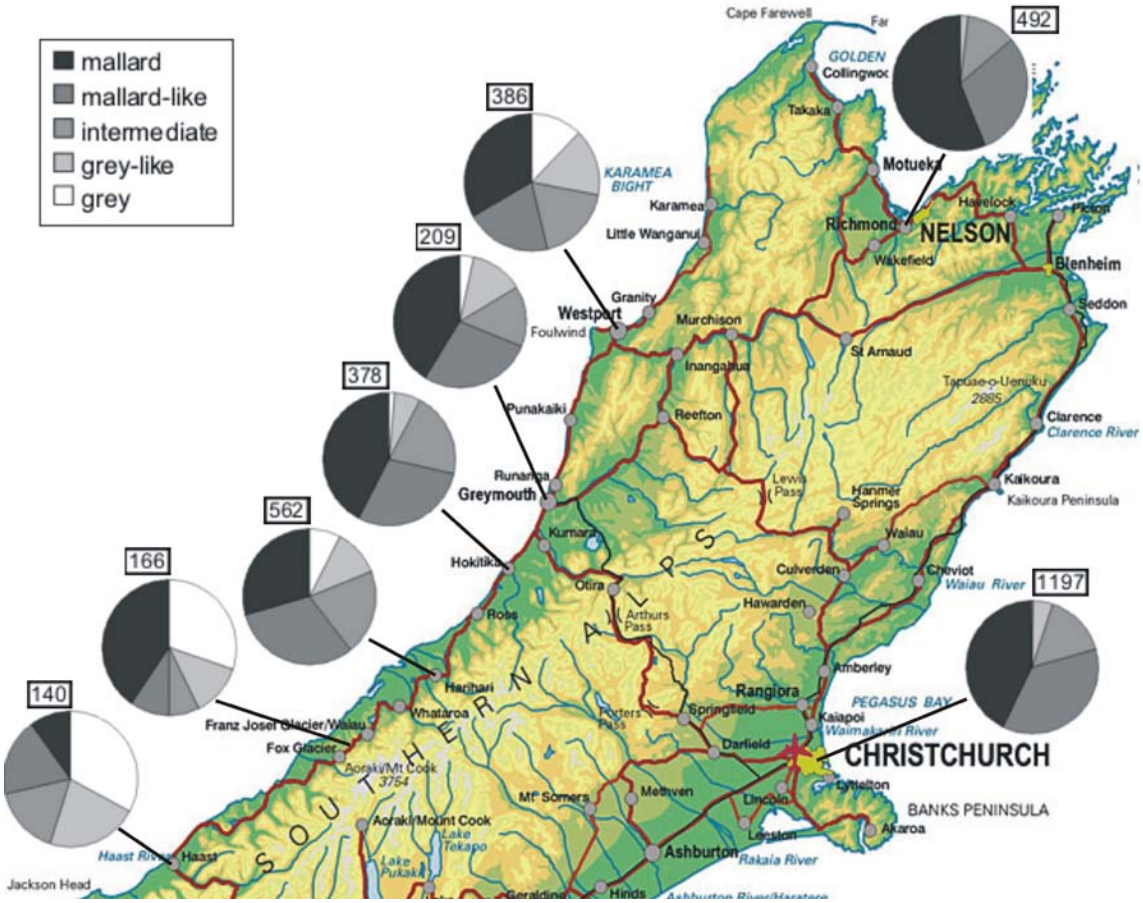

Figure 1. Map showing sampling locations and geographic distribution of mallard and grey duck phenotypes as well as their hybrids. The number at each pie chart indicates the number of individuals contributing to each chart.

Despite this general pattern, a more complex pattern emerges when each sampling location is considered separately (Table 2). Especially noteworthy is the greater than expected contributions of grey ducks in the area around Haast and the Glaciers and a similar but smaller excess of grey ducks in the Westport area. In contrast, a lower than expected number of grey ducks is seen in Christchurch and Nelson. Grey duck-like hybrids were also more numerous than expected in Westport and Haast, but rarer than

expected in Nelson and Christchurch. Intermediate hybrids appear fairly evenly distributed, but mallard-like hybrids were fewer than expected in Westport and the Glaciers, and more common than expected in Christchurch. Mallards were overrepresented in Nelson, and under-represented in both HariHari and in Haast (Table 2).

Table 2. Distribution of mallard, grey duck and their hybrid phenotypes in the eight locations surveyed, with row and column totals. Both sexes combined.

location	mallard	mallard-like hybrid	intermediate hybrid	grey-like hybrid	grey duck	total
Nelson	276	148	59	9	0	492
Westport	128	79	71	61	47	386
Greymouth	86	58	31	27	7	209
Hokitika	160	110	79	24	5	378
HariHari	165	176	115	64	42	562
Glaciers	67	16	12	21	50	166
Haast	14	26	23	31	46	140
Christchurch	511	440	186	58	2	1197
total	1407	1053	576	295	199	3530

These patterns held when I analysed the data for 2006 ($\chi^2 = 458.1$, df = 28, $p < 0.001$) and 2007 separately ($\chi^2 = 494.8$, df < 28, $p = 0.001$). In both analyses several expected values were below 5, limiting the reliability of the test. However, both tests remain significant if the contributions resulting from these values are excluded. On the whole, the pattern of contributions of each sample location is similar to that of the analysis of the

combined dataset. In 2006, the grey-like hybrid category was closer to expected and therefore shows no unusual contribution in Westport, Haast, or Christchurch, but there were fewer grey-like hybrids than expected in Greymouth. In 2007, there were fewer grey-like hybrids than expected at the Glaciers, but in Westport the level of grey ducks was almost as expected (results not shown). Differences between the two years were small enough that the combined dataset provides a relatively reliable estimate of the current distribution of the two species and their hybrids in the areas surveyed.

Comparison with historic data

As surveys in the historical record counted grey-like hybrids as pure grey ducks, and therefore overestimate the number of grey ducks, the proportion of actual pure grey ducks was likely to be lower historically than the data would suggest. Nevertheless, a comparison of my data with the historical data shows that the combined number of pure grey and grey-like ducks has decreased in all areas, and there is no sign that the trend is halting or levelling off (Figure 2).

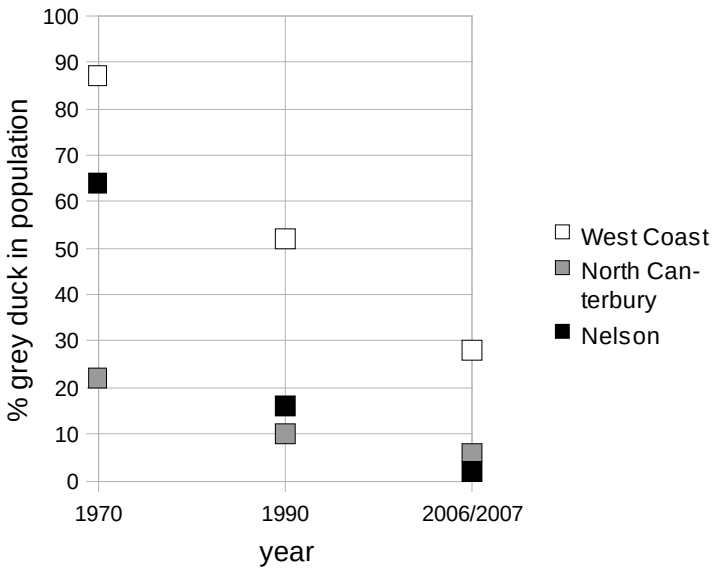

Figure 2. Changes in proportion of grey ducks in three districts over time. Data show percentage of grey and grey-like hybrid ducks in the total population (the remaining are made up of mallards and mallard-like hybrids, no intermediate hybrids are included). Data from 1920 and 1990 from Williams [1998] only recognise mallard-like and grey-like as categories.

Habitat segregation

The 304 ducks surveyed in the Haast and Glaciers area of the West Coast were not distributed randomly between habitats ($\chi^2 = 37.3$, df = 8, $p < 0.001$). Instead, mallards were found more frequently in natural habitats, with only few seen in human-dominated habitats, while grey ducks occurred in both habitat categories about equally (Figure 3). Hybrids were intermediate in their distribution in the different habitat types.

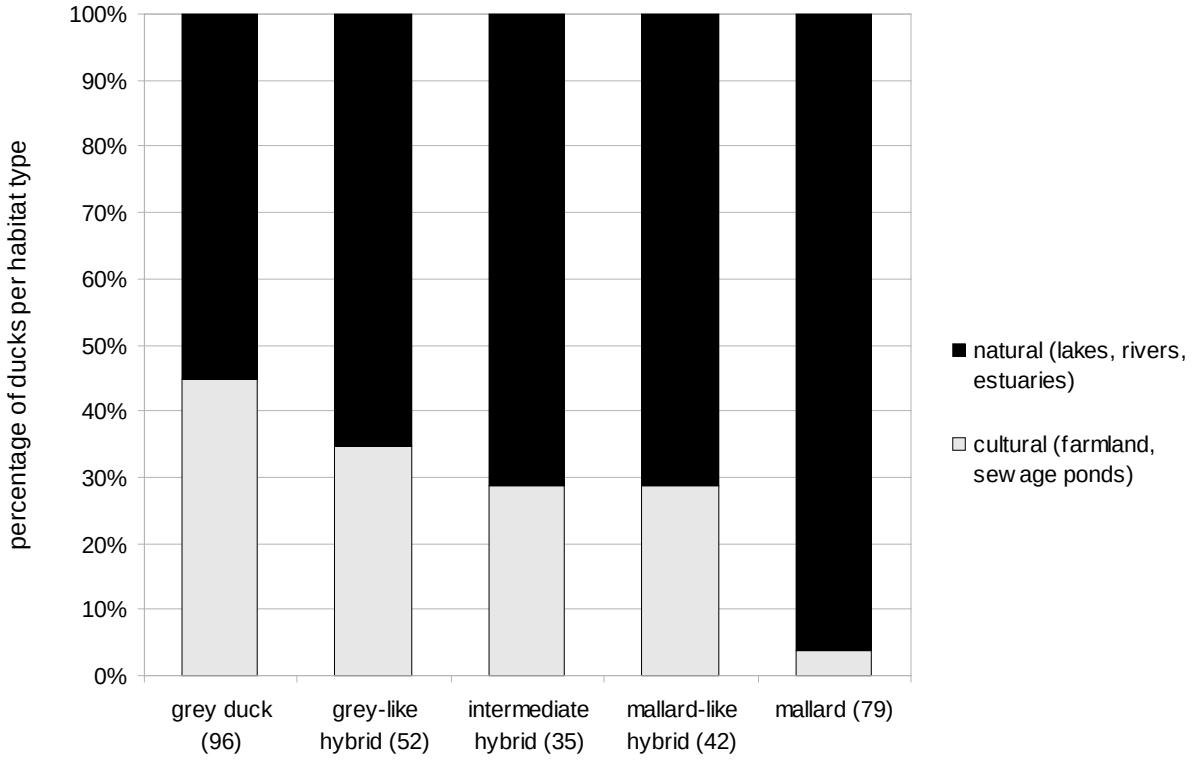

Figure 3. Occurrence of grey ducks, mallards, and their hybrids sorted for habitats disturbed by human influence (farmland, sewage ponds) and natural habitats (lakes, rivers, estuaries). Numbers by each category denote sample size. Total 304 individuals.

Discussion

My results suggest the historic decline of grey ducks in New Zealand has continued since the last comprehensive surveys. No ducks of a pure grey duck phenotype were seen in Nelson and only two in Christchurch. On the West Coast of the South Island, about 10% of ducks were identified as pure grey ducks, and almost all of these ducks were south of Franz Josef (43° 15' S). At the same time, more than 50% of ducks observed in

this region were clearly identified as hybrids. In all areas sampled, mallards and hybrids were now more common than pure grey ducks.

These results strongly suggest that the remaining grey duck population will in future shrink further as it disappears into the growing hybrid population, and that the integrity of the grey duck is imperilled. Given that hybrids interbreed with grey ducks and mallards (see next chapter), it stands to be expected that the hybrid population will grow and the pure grey duck population as well as the pure mallard population will shrink until they disappear.

Limitations of the study

All data presented here are based on the phenotype of ducks only. It has been noted that hybrids between mallards and grey ducks in captivity do not always display phenotypic characters intermediate between the parental types [Phillips 1921, Braithwaite and Miller 1975]. By investigating captive bred individuals of known hybrid status it has been shown that it can be difficult to accurately predict genotype from phenotype [Williams 1998]. In particular, mallards and mallard-like hybrids can be hard to tell apart, and a discriminatory function was unable to separate grey-like hybrids from grey ducks [Williams 1998]. In fact, phenotypic scores only offer a rough estimate of actual underlying hybrid status for many species pairs [Wilson 1992, Allendorf et al. 2001, Bronson et al. 2003]. Both overestimates and underestimates of the true values may ensue [Bensch et al. 2002, Randi and Lucchini 2002, Thulin et al. 2006]. In addition, the hybrid scores used here [Rhymer et al. 1994, see appendix] define the presence of any black colour in the bill as a sign of admixture with grey duck. While this is probably a reliable

criterion for males, mallard females commonly display black spots on their bills both in Europe and North America [Audubon 1967, Cramp and Simmons 1977, Marchant and Higgins 1990, Heather et al. 2000, Drilling et al. 2002]. Some characters, such as the strength of an eye stripe or leg colouration, might also be subject to interpretation and be classified differently by different researchers (pers. obs.).

Nonetheless, while hybrid scores may be of limited use to assess the hybrid status of any particular individual, I believe they can still be helpful in gaining general insights into trends and population status even if the actual levels of genetic introgression might not correspond exactly. In other words, phenotypic surveys that reveal widespread hybridisation can still provide valuable evidence of a potential conservation problem even if the exact details of the underlying processes are not clear. Indeed, surveys based on phenotype are likely to remain an important tool in studies of interspecific hybridisation, as it is difficult, time consuming and expensive to perform large scale molecular studies (for further data, validation and a discussion of the phenotype and genotype link in this species pair, see also chapter 4).

Critically for this study, the most important number, the number of grey ducks, is much more likely to be over- rather than underestimated, since a small proportion of mallard genes is likely to be invisible, while it is less likely that grey ducks are grouped as hybrids. Such cryptic hybrids are common in other species pairs [see e.g. Pfenniger et al. 2002, Babik et al. 2003, Chan et al. 2006]. This may mean the number of genetically pure grey ducks in New Zealand is probably even lower than my figures would suggest.

Like many previous studies of hybridisation in grey ducks and mallards [Williams 1998], I also found a surplus of mallard-like hybrid females, and a lack of mallard

females [Williams 1998]. It is normal in mallard populations that males outnumber females [in North America ~1.33 males per female, Drilling et al. 2002], but it is more difficult to explain a larger number of mallard-like females. While a surplus of mallard-like hybrid females could arise because certain plumage types are more likely in this cross, or because there is sexual selection for mallard phenotype males, it is most likely that it can be attributed to a probable misidentification of mallard females as mallard-like hybrids [Williams 1998], due to their variable colouration [Cramp and Simmons 1977, del Hoyo et al. 1992]. Undoubtedly the accurate assessment of hybrid status is more difficult in females than males, as the females of both species are similar in appearance. As an added problem, as mentioned above, the plumage score used here and in other studies specify that any dark colouration in the bill of a female is a sign of hybridisation, while female mallards commonly show such partly black bills [Audubon 1967, Cramp and Simmons 1977, Marchant and Higgins 1990, Heather et al. 2000, Drilling et al. 2002]. Removing this criterion alone, at least for females, might rectify discrepancies in future studies.

Spatial distribution of hybridisation and current status of the grey duck

Findings presented here support the results of earlier studies, namely that the grey duck phenotype is rare on the East Coast and Nelson areas, and while they are also declining on the West Coast, they are still more common there than anywhere else. In my survey, not only did I confirm the low numbers of grey ducks outside the West Coast, but I failed to find any ducks of a grey duck phenotype in Nelson, and so few in Christchurch that their genetic status is highly doubtful. Even on the West Coast, only 10% of ducks

are of grey duck phenotype, and less than half that in most locations. Based on these results, it may be time to reassess the national status of the grey duck in New Zealand, which is currently considered as least concern by the IUCN.

The spatial pattern of hybridisation: habitat hypothesis

Three hypotheses have been put forward to explain why mallards are replacing grey ducks in New Zealand [Williams and Basse 2006]. These are that (1) mallards outcompete grey ducks by greater survival, fertility, and competitive ability, (2) that mallards assimilate grey ducks by hybridisation, and (3) that human-modified habitats are more suitable for mallards than grey ducks. The latter hypothesis has led to the prediction that the grey duck populations at the West Coast will remain stable despite the presence of mallards, since natural habitat is more common there than elsewhere on the South Island. While grey ducks are often said to prefer remote watercourses, rivers with natural vegetation, and ponds in the forests, mallards have been proposed to favour farmland, cities, and open water around settlements [e.g. Heather et al. 2000]. My data do not confirm this, and indeed rather suggest the opposite – mallards occurred more often in natural habitats, while grey ducks occurred in both natural and disturbed habitat in about equal frequencies. In any case, many habitats such as large lakes and estuaries are shared by the two species, and grey duck populations have vanished elsewhere in natural habitats, with mallards and hybrids invading readily, and the theory has therefore been dismissed previously [Williams and Basse 2006].

The spatial pattern of hybridisation: a new, temporal hypothesis

I believe that the spatial spread along the West Coast can instead be explained as a temporal pattern. Though mallards can live at higher altitudes, they usually prefer lowlands, and stagnant or slow flowing water [Cramp and Simmons 1977, del Hoyo et al. 1992]. They are rarely seen in the fast flowing mountain streams of the Southern Alps, and though they are capable of crossing mountain chains, the Alps may act as a barrier to dispersal. Data in the surveys quoted above suggest that mallards colonised the West Coast only around 1970, twenty years after they became abundant in Otago and the Canterbury Plains. This delay in colonisation of the West Coast is also supported by the appearance of first male mallard hunting trophies on the West Coast at this time (pers. communications). It is possible mallards first reached the West coast by crossing the Southern Alps in the north (near Westport) where mountains are lowest, and colonised the farmland and other habitats between and around Westport and Hokitika, where hybrids appeared in their wake. They then spread southwards along the coast, reaching the Glaciers and Haast areas a decade or so later. This would mean that any development south of the Glaciers lags about thirty years behind those on the East Coast.

Conclusion and future outlook for the grey duck

If the spread of mallards is mostly the product of a time lag, rather than a reflection of habitat quality, then the larger number of grey ducks seen on the West Coast, and at its southern part in particular, may not reflect any advantage for grey ducks over mallards that may be due, for example, to the more natural habitat in this region. Instead, it might simply reflect an earlier stage in the same process of mallards replacing grey ducks as

seen on the East Coast several decades previously. If this scenario is correct, then in another twenty years time, grey duck phenotypes will be as rare on the West Coast as they are on the East Coast now, with a mallard and hybrid swarm making up the entire duck population of New Zealand, and the grey duck extinct in this country.

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Chapter 3

Like with like: assortative mating in mallards, grey ducks and their hybrids in New Zealand

Abstract

A survey of assortative mating among mallards (*Anas platyrhynchos*), grey ducks (*A. superciliosa*) and their hybrids was conducted by surveying pairs of birds along the West Coast of the South Island, and in the cities of Nelson and Christchurch, New Zealand. Phenotype was used to classify individuals as either hybrids or one of the two parental species, and paring status was determined by proximity and behavioural cues. Among a sample of 987 ducks, no pairs were found between phenotypically pure grey ducks and pure mallards. Both mallards and grey ducks were most likely to pair with others of their own phenotype. Likewise hybrids mainly paired with other hybrids of a similar phenotype. The latter pattern was weaker than the preference of parent species for their own phenotype, and might arise because hybrids are rejected by either parental species as partners. Although a general pattern of assortative mating was found among all hybrid classes, there was a tendency for females, regardless of her phenotype, to pair with males more mallard-like than themselves. This apparent preference for male mallard phenotypes may promote continued hybridisation, and combined with assortative mating within hybrids, might explain the fast growth of the hybrid swarm, and increasingly mallard-like appearance of the hybrid population in New Zealand.

Introduction

Large scale interspecific hybridisation is gaining recognition as a serious conservation problem that can lead to the extinction of species via a loss of genetic integrity [Rhymer and Simberloff 1996, Allendorf et al. 2001, Mooney and Cleland 2001, Levin 2002, Perry et al. 2002]. Humans can induce or increase hybridisation in various ways, including habitat changes, or the introduction of species into areas from which they were previously absent [Hendry et al. 2006, Seehausen 2006, Seehausen et al. 2008].

The mallard (*Anas platyrhynchos*) is one species for which hybridisation has been recorded extensively. To date, the mallard is known to hybridise with 23 other species of dabbling ducks [Marchant and Higgins 1990, del Hoyo 1992]. However, in most cases this hybridisation does not lead to high levels of introgression and both parent species usually remain separate [e.g. Peters et al. 2007]. Exceptions occur particularly where the mallard has spread or been introduced into the ranges of previously allopatric species of dabbling ducks. For example, the introduced mallard in New Zealand hybridises widely with the native grey duck (*Anas superciliosa*), threatening the integrity of the latter species [Dorst 1970, Drilling et al. 2002].

Mallards were first imported into New Zealand about 1870 but only became firmly established when released in greater numbers between 1930 and 1960 [Knox 1969, Heather et al. 2000]. It is estimated that in 1960 grey ducks made up 95% of all New Zealand dabbling ducks, but by 1985 that proportion had declined to 20% and they were outnumbered by hybrids (25%) [Marchant and Higgins 1990]. Regionally, the development was even more extreme. In Otago less than 3% of the ducks appeared to be hybrids prior to 1958, but by 1981-82, they made up 51% of the population [Gillespie

1985]. The most recent estimates from 1991 indicate that 41% of the national population were hybrids [Williams 1998]. To understand the underlying dynamics of this hybridisation and to predict the population's fate, an understanding of the mating patterns within the population is crucial.

Even in populations where hybrids occur occasionally, mating is often assortative. In general terms, assortative mating is simply the mating of individuals with similar phenotypes or genotypes. For example, despite some gene flow between introduced sika deer (*Cervus nippon*) and native red deer (*C. elaphus*) in Scotland through hybridisation, both species mate preferentially with members of their own species, and they remain two distinct and recognisable populations [Goodman et al. 1999]. The naturally co-occurring Darwin's finches, *Geospiza scandens* and *G. fortis*, also will hybridise occasionally, but remain two distinct species even though gene flow can be substantial at times [Grant et al. 2004]. In the contact zones of hybridising populations of collared (*Ficedula albicollis*) and pied flycatchers (*F. hypoleuca*) only about 4% of pairs are mixed, but 13% would be predicted if mating was entirely random [Atalato et al. 1982], suggesting that individuals either avoid heterospecifics or prefer conspecifics. In other cases, mating does appear random, as observed in some trout (*Oncorhynchus clarki lewisi* x *O. c. bouvieri*) [Gyllensten et al. 1985] and tiger salamander (*Ambystoma californiense* and *A. tigrinum*) populations [Riley et al. 2003]. Sometimes, mating may even be disassortative. For example, females of the Pecos pupfish (*Cyprinodon pecosensis*) prefer males of the sheepshead minnow (*C. variegatus*) over their own males, accelerating hybridisation [Rosenfield and Kodric-Brown 2003].

As these examples suggest, mate choice may be an important factor that can either promote or limit the formation of hybrid populations. The role of mate choice in the formation of hybrid populations depends not only on the mating preferences of each of the parental species, but also on the preferences and mating success of fertile hybrids. Some hybrids are sexually fully competitive [e.g. hybrids of blue-winged and golden winged warblers (*Vermivora pinus* x *V. chrysoptera*), Vallender 2007], but more commonly there is sexual selection against hybrids [e.g. collared and pied flycatchers (*Ficedula albicollis* and *F. hypoleuca*), Svedin et al. 2008; Antarctic, Subantarctic and New Zealand fur seals (*Arctocephalus tropicalis* and *A. forsteri*), Lancaster et al. 2007]. Unfortunately, the mating preferences of hybrids themselves have rarely been measured although being potentially crucial for the fate of hybrid populations (see discussion).

Details of mallard mating behaviour make it reasonable to investigate if sexual selection might play a role in the hybridisation with grey ducks. While male mallards court females of their own and other species [McKinney 1983, Seymour 1990], females are much more discriminating. Female mallards have been shown to prefer males with bright yellow bills [Peters et al. 2004, Omland 1996a, Omland 1996b], males with intensely coloured plumage [Klint 1980, Weidman 1990, Omland 1996a, Omland 1996b], males that moult early [Omland 1996a, Omland 1996b, Cunningham 2003] and males that have high testosterone levels [Davis 2002]. Female mallards also prefer males that most actively display to them [Williams 1983], and will themselves actively compete with other females for access to the most attractive males [Weidman 1990]. Some of these male signals are known to be honest signals that correlate with the quality of a potential mate. For example, a yellow bill reflects caretenoid levels, which predicts

immunocompetence and sperm motility [Peters et al. 2004]. Similarly, the preference for males with high testosterone levels and early moults appears to predict aggressiveness and the quality of mate guarding, which protects females from forced extrapair copulations [Davis 2002].

As female mallards thus appear to select males based on phenotypic characters, and the necessary cues are conveyed at least in part by genetic preferences, it is reasonable to expect that hybrid females may also exhibit some degree of preference for mallard phenotypic traits if they inherit that preference from the mallard parental species. On the other hand, the mate choice strategies of female grey ducks are less known, but it has been assumed that colouration is less likely to play a role since both sexes are relatively dull-coloured and monomorphic [Heather et al. 2000]. It is therefore not unreasonable to suggest that there might be a degree of mate preference for males with mallard-like phenotype, not only among pure female mallards but also amongst hybrids. This should in turn lead to some degree of positive assortativeness in mating, with mallard females preferring mallard males, grey duck females preferring grey duck males, but with female hybrids perhaps showing a preference of mallard and mallard-like males.

In this study I set out to determine the degree of mating assortativeness in wild populations of mallard, grey duck and their hybrids. The expectation was that mating would be non-random, but assortative within each parental species, and perhaps among hybrids as well. Furthermore, if mate choice preferences for more colourful males have a genetic basis and are at least sometimes present in hybrid females, then hybrid males resembling mallards would be expected to be preferred by hybrid females over more grey-like hybrid males.

Methods

Survey data were used to test the presence of assortative mating among mallards and grey ducks. The phenotypes of males and females in pairs observed in the wild were recorded, and compared to the pairs expected if mating was random with regard to phenotype within each of the surveyed populations. While this method might suffer from inaccuracies in the identification of the phenotype of an individual, or mistakes in pairing status, it does enable the study of pairing patterns in the wild rather than in an artificial environment, and thus is more indicative of what might explain the current levels of hybridisation between the two species in New Zealand. However, it should be noted that such surveys by themselves do not directly demonstrate mate choice preference for phenotype, but instead I have assumed that the two birds associating in a pair do so because of such a preference. In other words, I have assumed that an association between two birds (i.e., birds observed as a pair) is the result of mating preference. Testing this assumption would require captive breeding experiments, and was beyond the scope of this study.

Two surveys were conducted, one in late June 2006 and the other in early July 2007. At this time of the year, both species have already formed pairs, but most females have not yet laid eggs [Heather et al. 2000], making pairs more obvious (pairs are more difficult to determine after eggs are laid as females spend much time incubating). On both surveys the weather was cold, frosty and sunny, offering excellent visibility. Binoculars were used where necessary.

Study locations

Surveys for ducks were done across a series of eight locations. The locations spanned a north-south trajectory along the West Coast of the South Island of New Zealand, from Nelson to Haast, at approximately equally distant intervals. These were Nelson (41° 17' south, 173° 15' east), Murchison (41° 47' south, 172° 19' east), Westport (41° 45' south, 171° 37' east), Greymouth (42° 27' south, 171° 12' east), Hokitika (42° 44' south, 170° 58' east), HariHari (43° 8' south, 170° 34' east), the Glaciers (both Franz Josef, 43° 21' south, 170° 10' east, and Fox, 43° 28' south, 169° 59' east, areas combined), and Haast (43° 52' south, 169° 2' east). Christchurch (43° 32' south, 172° 37' east) was used as an example of the current population structure on the east coast. Because low numbers of ducks were recorded in Murchison (N=18), this location was dropped from the analysis and not surveyed in the second year. Each location was surveyed for one day, beginning shortly after sunrise until late afternoon. I searched all suitable locations such as lakes, rivers, ponds and sewage treatment works in an area of approximately 5-10 km around each of the 8 locations. Sites to search were identified beforehand using maps and satellite pictures.

Categorisation of species and hybrids for phenotype

Each site was systematically searched and all ducks observed were classified as belonging into one of the following five phenotypic categories: mallard, mallard-like hybrid, intermediate hybrid, grey duck-like hybrid, or grey duck. A previously developed plumage score [Rhymer et al. 1994, see appendix] was used as a guideline, but some diagnostic body parts (e.g. legs) were not always visible. All visible criteria, including

body colouration, were used when assessing the status of each individual. Body colour of male mallards is grey with maroon breast, female mallards are brown with feathers edged by wide light margins, and grey ducks of both sexes have dark grey body feathers with small light edges. To be classified as either a pure mallard or pure grey duck, an individual could not display any signs of admixture with the other species. Ducks with some signs of hybridisation, for example orange feet in an otherwise grey duck phenotype, or lack of green on the head in a male mallard, were assigned to the mallard-like or grey-like categories. Intermediate hybrids were those birds intermediate between the two types, but not noticeably more similar to one side than the other.

Identification of pairs

Wherever courting behaviour was observed, or two birds associated in a way characteristic of pairs (see below), the individuals were assumed to be pairs, and their phenotype noted. In mallards, pairs are known to behave notably different from unpaired individuals particularly in keeping in close proximity to their partners but apart from other ducks [Bauer and Blotzheim 1968]. The courtship and pair bonding behaviour of mallards and grey ducks is thought to differ little [Marchant and Higgins 1990]. Displays typical of pair bond maintenance included inciting by the female, mock preening such as preen-behind-the-wing or preen-dorsally, bill-dipping or ceremonial drinking, as well as *raeb-raeb* calls in chin-lift posture by the male or both [Bauer and Blotzheim 1968, Cramp 1977, Marchant and Higgins 1990, del Hoyo 1992]. These elements are often combined. There is also mutual forward stretching in greeting of partners and the defence of a female by a male via attacks on other males (i.e. mate guarding) [Bauer and

Blotzheim 1968, Cramp 1977, Marchant and Higgins 1990, del Hoyo 1992]. To be classified as a pair without such displays, the ducks had to sit, walk or swim together, and if stationary they had to be in less than 50 cm proximity, with no other ducks within a 1 meter radius around either duck. If in motion they had to move in the same direction and be within 1.5 m of each other, provided no other ducks were within a radius of ~4 m around either duck. Any antagonistic behaviour between the two partners was considered a sign that there was no stable pair bond [Bauer and von Blotzheim 1968], and such birds were not considered pairs. As I only watched each putative pair for periods of less than 10 minutes, it is possible that some associations I observed were not true pairs and only remote associations. However, given the known tendency of males to intensively guard their mates in most waterfowl, including mallards, it is likely that most associations of two birds during the pairing season were in fact pairs.

The sex of each bird was recorded when possible. The sex of mallards was easily determined by plumage, such as body colouration and features such as the curled tail feathers of drakes. Most hybrids could also be sexed on the parts of their phenotype that was characteristic of mallards. However, since grey ducks are monomorphic, all grey ducks and some grey-like individuals could not be assigned to a sex in this way. In some cases, courting behaviour allowed a clear assignment. The remaining cases were marked as unknown sex.

Statistical methods

Statistical tests were performed using Excel spreadsheets and Minitab (version 15.1.0.0). For count data, χ^2-tests were performed to investigate most questions. A

preliminary data check revealed significant differences between the data collected in the two years (χ^2 = 30.8, df = 4, p<0.001). Accordingly analyses were performed for each year separately as well as on combined data to assess potential discrepancies in results. To control for geographically differing population structures (see Chapter 2), the numbers of pairs expected if mating was random were estimated within each population separately, and then combined to estimate how many pairs of each type should have been encountered in the entire survey if mating was random within the population at each location. Expected values for each location and possible pairing of plumage types were compared with a χ^2-test, using the formula: (number of females of one plumage type/total number of females)*(number of males of one type/total number of males) * (total number of pairs). The expected values for all locations were added together for the analysis. This method takes into consideration local heterogeneity, as it would have been unrealistic to treat the entire South Island population as one homogeneous one. For example, it is more unlikely for a grey duck in Christchurch to find the one or two other grey ducks in hundreds of individuals than it is for a grey duck in Haast to find another grey duck, as they make up a third of the population there (for data see Chapter 2).

The number of female and male ducks varied significantly between categories when data for both years were combined (χ^2 = 44.6, df = 4, p<0.001). For example, there were more male than female mallards, and more female than male mallard-like hybrids. There was also a slight surplus of grey duck females, but since grey ducks cannot always be reliably sexed, this result could be an artefact. There also appears to be a general surplus of males in the population, with 28.4% more males than females (for raw data, see previous chapter).

Of the 987 pairs recorded, 17 were between a grey duck and a grey-like hybrid in which it was impossible to assess which partner was the male and which the female. All tests were run four times: once excluding all doubtful pairs, once including them but assigning half the pairs to where the grey duck was the male and half to where the grey duck was the female, once assuming the grey duck was always the male, and lastly assuming that the grey duck was always the female. Differences between these four analyses were small and the tests shared patterns of significance, therefore only the analysis of the dataset assuming half the ducks of unknown sex to be male and half of them to be female are shown.

Results

Raw data

Table 1 details the phenotypes of all 987 pairs seen in the survey. Of these, 434 were observed in 2006, and 554 in 2007. Pairing between apparent pure phenotypes (i.e., pure mallard with pure mallard, and pure grey duck with pure grey duck) comprised only about a third of all pairings (294 or 29.9%). All remaining pairings involved hybrid birds. Pairs expected if mating was random within all ducks present at each location are also listed in Table 1.

Table 1. Matrix of phenotypes of female and male ducks recorded in 987 pairs (obs), and numbers expected if mating were random within each location (exp). *Pairs of grey ducks with grey-like ducks where it was unknown which one was of which sex appear as half each (17 pairs). Shading indicates how much the female and male of a pair differed with regard to phenotype. Key below.

	female									
male	**mallard**		**mallard-like hybrid**		**intermediate hybrid**		**grey-like hybrid**		**grey duck**	
	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
mallard	239	153	154	88	48	52	8	25	0	13
mallard-like hybrid	59	152	133	94	66	56	8	26	0	12
intermediate hybrid	22	72	46	45	46	28	8	16	1	9
grey-like hybrid	2	29	12	19	8	13	46	10	16.5*	8
grey duck	0	22	0	14	0	11	8.5*	10	56	11

same category	differ by 1 category	differ by 2 categories	differ by 3 categories	grey duck and mallard

Assortative Mating

A test comparing observed proportions of pairs with expected proportions confirms that mating is non-random ($p < 0.001$, $\chi^2 = 680.36$, df = 16). As a comparison between expected and observed values in Table 1 shows, mating appeared to be highly assortative. Figure 1 summarises these data, and clearly shows that pairs comprising males and females of the same phenotype were observed more frequently than expected (1.68 times as often as expected by chance), and mixed pairs were observed less frequently than expected. The more the partners differed in phenotype, the rarer were the pairings seen.

No pairs were observed between a phenotypically pure mallard and a phenotypically pure grey duck.

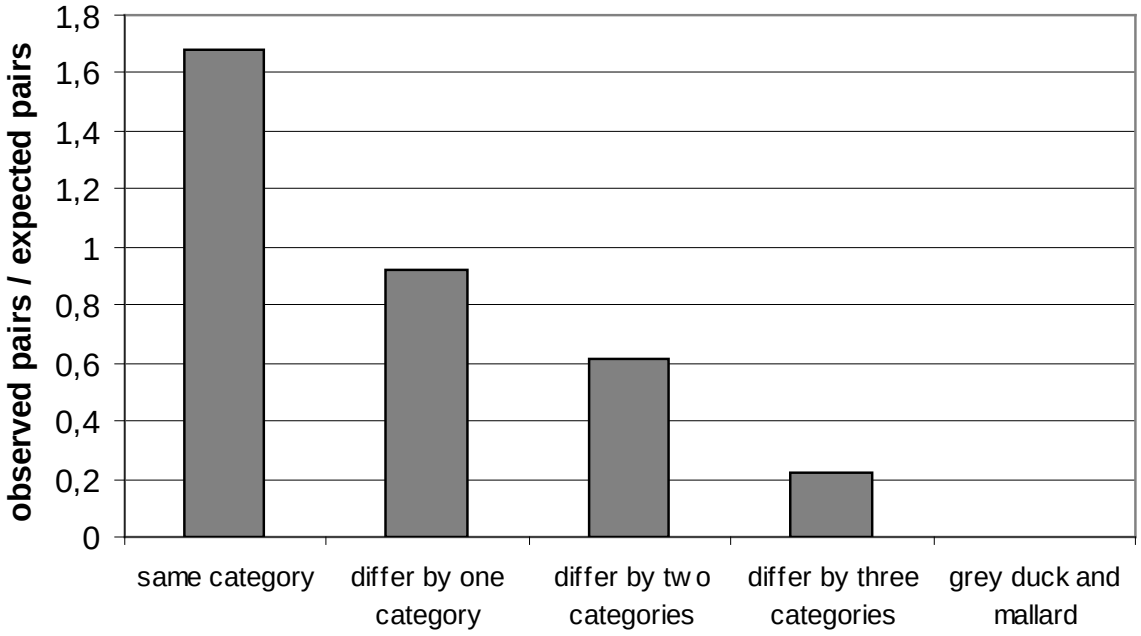

Figure 1. Ratio of observed pairs / expected pairs, sorted by phenotypic differences between the two ducks in the pair. Individuals were classified in five phenotypic hybrid categories (mallard, mallard-like hybrid, intermediate hybrid, grey-like hybrid, grey duck). Sample size is 987 pairs. No pairs of a pure grey duck with a pure mallard were observed.

Pairings between two pure grey ducks, and those between a male and female grey-like hybrid were both more numerous than expected by chance, and the females of either were rarely seen with mallards or mallard-like males. Likewise, pairs between mallard females and mallard males were more numerous than expected by chance, while pairs of mallard females with mallard-like males, hybrid males, grey-like hybrid males and grey duck males were all less common than expected. Mallard-like females showed a surplus of

pairings with mallard males and mallard-like males, but were less likely than expected by chance to pair with grey males, whereas intermediate hybrid females appeared to pair with mallard, mallard-like, or grey-like males about as often as expected by chance, but paired more often with other intermediate hybrid males than expected, and more rarely with grey ducks. However, the contributions of these values are small, so the affinity is much less pronounced than in the two pure phenotypic groups. Overall, each female was most likely to pair with males of her own phenotypic class.

Mate choice for mallard phenotypic traits?

Although the overall pattern I found was for individuals to pair up with another individual of the same or similar phenotype, I tested if there was a tendency for females to pair with mallards or mallard-like individuals by comparing in more detail the phenotypes of pairs in which partners were from different phenotypical categories. The chance of pairs forming between a female and a male one or two categories more mallard-like than herself were about double that of a female choosing a partner that was one or more categories more grey-like than herself (Figure 2). In pairs differing by three categories, which were either a grey duck female and a mallard-like male or a grey-like female and a mallard male, the female was always the grey or grey-like individual. On average, the male of a pair was phenotypically more mallard-like than the female ($p < 0.001$, $\chi^2 = 118.04$, df = 2). A lack of pairs in which the male was more grey-like than the female was responsible for this result, rather than a surplus of pairs in which the male was more mallard-like than the female.

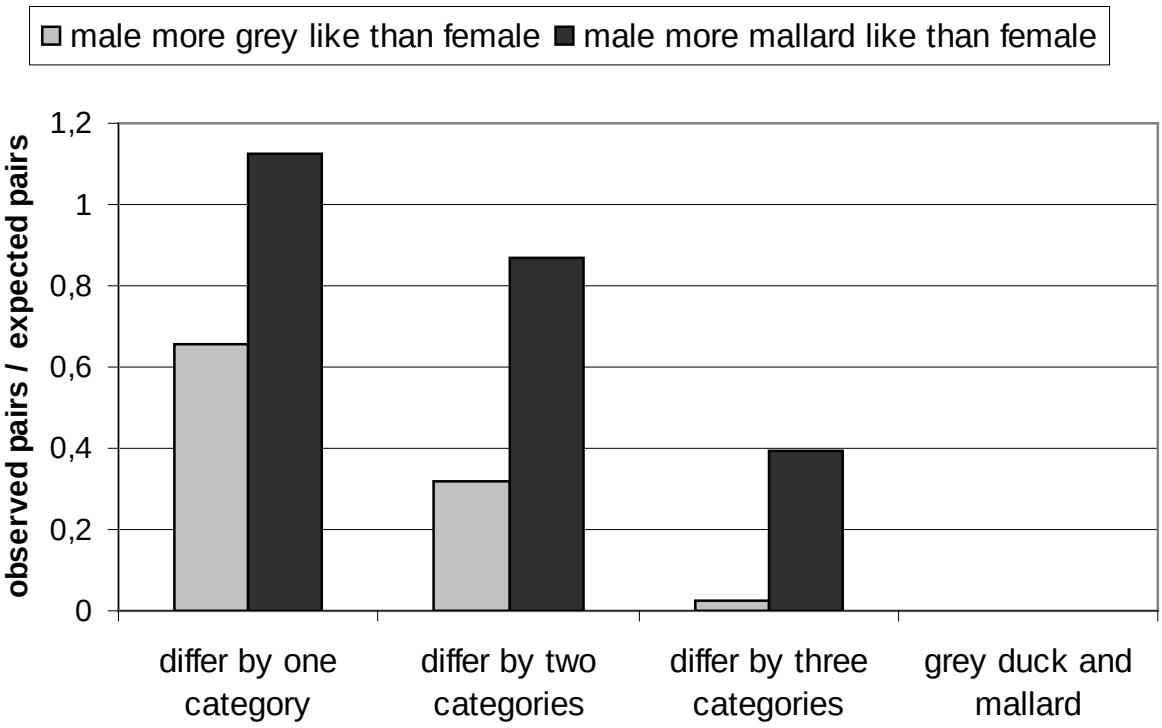

Figure 2: Ratio of observed pairs / expected pairs, sorted by phenotypic differences between the two ducks in the pair, and comparing pairs where the male was more mallard-like than the female, or vice versa. Individuals were classified in five phenotypic hybrid categories (mallard, mallard-like hybrid, intermediate hybrid, grey-like hybrid, grey duck).

Discussion

I found that despite widespread hybridisation, as expected both grey ducks and mallards mated assortatively by phenotype. Even amongst a sample of almost a thousand pairs of ducks, I did not observe any hybrid pairings between pure individuals of either parental species. However, pure pairs within each parent species comprised only about a third of all observations with the remaining pairings being between birds of varying hybrid status. More surprising was an apparent, if weaker, trend of hybrids to mate with other hybrids.

One possible reason for the extensive hybridisation might be a mating preference by grey ducks or hybrids (of either sex) for mallard or mallard-like phenotypic traits. I found some evidence for this in the over-representation of grey-like females pairing with males that were significantly more mallard-like than themselves.

Limitations of the study

The results presented here are based on the phenotype of ducks only. Phenotype is only a rough indication of genotype [see previous chapter for discussion], and therefore, individuals might have been misclassified in regard to their actual hybrid status at a genotypic level. In particular, the distinction of mallards and mallard-like hybrids by phenotypic characters alone in females is probably unreliable [Williams 1998]. For further data, validation and a discussion of the phenotype and genotype link in this species pair, see also chapter 4.

However, as mallard females themselves are known to use phenotype traits when selecting potential mates [Peters et al. 2004, Omland 1996a, Omland 1996b, Weidman 1990, Klint 1980], it seems reasonable to expect that patterns of pairing based on phenotype should reflect at least partly an individual's mate choice decision. Despite the apparent tendency for females to prefer mallard males, it is not clear if the apparent advantage in mate choice experienced by male mallards drives the population towards an actual dominance of the mallard genome, or if only genes that encode phenotypic characters conveying male sexual fitness are selectively favoured. It is known that in hybridising populations, genes conferring advantages can introgress and sweep to fixation rapidly [Martinsen et al. 2001].

Comparison with previously published data

No single pairing between ducks of opposing pure phenotypes was observed in this study, and mating in general was found to be assortative to a lesser degree within all five hybrid categories. A previous study found pairs between grey ducks and mallards to be rare in Waikato [~4%, Hitchmough et al. 1990], but the data are from 1967-68 and no hybrids were recognised, so it is possible that some pairs involved mallard-like or grey duck-like hybrids. One explanation for the assortativeness in pairing I observed may be due to the asynchronous if overlapping mating seasons of the two species. Mallards mate in late August to early October, but grey ducks in late September to early December [Williams and Roderick 1973], and thus the opportunities for parental species to hybridise via the formation of pairs are limited to the narrow overlap period. Sperm storage might slightly increase the time in which male mallards could produce offspring with grey duck females, but little is known about sperm storage in female grey ducks. However, mallards in good habitat and condition tend to extend the mating period, with chicks in cities sometimes seen until late in autumn (pers. obs.). The overlap period lasts at least several weeks, and potentially longer. This might limit the formation of mixed pairs, but will not in itself be enough to prevent it. Another possibility is that grey duck males might be unattractive to mallard females since mallard females are known to prefer visual cues including yellow bills and males with green heads and maroon breasts, all of which are absent in grey duck drakes. It is also known that grey duck drakes do not display to mallard females at least in captivity [Williams and Roderick 1973].

It is harder to explain why pairs with a male mallard and female grey duck are rare. In ducks, as in many animals, females are usually the choosy sex, and males will even mate

with females of many other species that let them. Indeed, pairings between male mallards and female grey ducks have been reported more often than the opposite [Williams and Roderick 1973, Hitchmough et al. 1990]. Perhaps male mallards are less willing to permanently pair with a grey duck female then to display and copulate with her, or perhaps male mallards get rejected by grey duck females. It is also possible that female mallards outcompete grey duck females for the males they prefer. All of these possibilities require further study.

Possible causes for initial hybridisation

My results suggest that the high level of assortative mating may explain why both species are seen living side by side for a decade or two before hybrids appear in numbers. Normally, these species are separated by pre-mating isolation mechanisms (e.g. different if overlapping breeding seasons, different courtship behaviour). Theories that might explain why this barrier breaks down and first hybrids appear include forced copulations [McKinney et al. 1983, Seymour 1990, Randler 2005], egg dumping resulting in imprinting of males on the wrong species' females [Randler 2005], or mate choice mistakes [Randler 2002]. Forced copulations and egg dumping are relatively common in ducks and thus might be the mechanisms that initiate hybridisation. Indeed, both could co-occur with a male duck misimprinting on a "forster mother" of the other species as a result of egg dumping, and which might then later force copulations on females of the other species. There are no estimates of the frequency of forced copulations in grey duck, but it is common in mallards and male mallards sometimes attempt forced copulations with females of other species [McKinney et al. 1983, Seymour 1990, Randler 2005].

However, a study of mtDNA in the hybrids of the two species shows that introgression is bidirectional [Rhymer et al. 1994]. At some stage, therefore, at least some pure mallard females must have been involved. These matings might have occured with hybrid males rather than with pure grey duck males, so these results are not evidence for matings between pure mallard females and pure grey duck drakes. It is probably too late, at least in the New Zealand population, to find out how initial hybridisation occurred.

Implications of assortative mating for temporal dynamic of hybridisation

By whatever mechanism rare hybrids arise, and assuming they are fertile and successful in gaining a partner, they tend to backcross to one parental population, with the backcross offspring again crossing back to that population. This results in the uni- or bidirectional transfer of genetic material, rather than a build-up of a noticeable hybrid populations [e.g. Goodman et al. 1999, Roques et al. 2001, Green and Parent 2003, Glor et al. 2004, Grant et al. 2004, Lancaster et al. 2006, Kingston and Gwilliam 2007]. On the other hand, if hybrids mate selectively with each other, a much faster growth of the hybrid portion of the population is to be expected.

The mating preference of hybrids for other hybrids that is suggested in my survey might be caused by a real preference – backcross males should be imprinted on their hybrid mothers, so may display mainly to hybrid females. It is also possible that hybrid females have a genetic preference for only some of the genetically encoded mating signals of mallard males, and quite possibly different signals in different hybrid females. Alternatively, there might not be a real preference at all, and hybrids may simply be rejected by pure individuals and therefore end up pairing each other. Each of these

possibilities cannot be tested with my data and experimental laboratory trials of mate choice would be needed to determine the exact traits preferred and their potential genetic basis.

Assortative mating of hybrids in other species

Few studies have shown assortative mating within hybrids, or even investigated the mating preferences of hybrids either of first or of subsequent generations, as opposed to the mating preference of the parental species. A notable exception is Mavarez et al. [2006], which demonstrated that hybrids of the butterflies *Heliconius melpomene* and *H. cydno* prefer other hybrids over either parental species, and in fact that a third species, *H. heurippa,* appears to have evolved from the hybrids of these two parental species. In the case of the grey duck and mallard, assortativeness within hybrids is not strong enough for such hybrid speciation in presence of the parental species, but it may well be a factor in aiding the development of a hybrid swarm, rather than two populations with limited introgression.

Sexual selection in hybridising populations

I found some evidence of a mating advantage for phenotypic mallard males. Sexual selection can lead to the rapid introgression of sexually important characters into a population. White-collared manakin (*Manacus candei*) females, for example, prefer the colouration of golden-collared manakin (*M. vitellinus*) males over that of their own males, and in response this trait spreads rapidly over the hybrid zone [Stein and Uy

2006]. How much genetic material beside the locus or loci encoding colouration introgress is open to speculation.

Previous studies have shown evidence of asymmetrical hybridisation in the species pair of grey duck and mallard, if only within what was reported to be pairs of the original species. In Waikato, for every five pairs of a mallard drake with a female grey duck, Williams and Roderick [1973] reported two pairs of mallard females with grey drakes, and Hitchmough et al. [1990] reported the more extreme ratio of 18:1 in the same area. Both studies, however, do not mention any hybrids as parental birds, and as it is unlikely that no hybrids of the previous years were present, it is probable that some individuals were not identified correctly. This limits the strength of the comparison considerably, but does support a trend for the male of a pair to be more mallard-like than the female.

The development of a mallard/grey duck hybrid swarm in New Zealand that is biased towards more mallard-like individuals has previously been observed and has variously been attributed to the fact that mallards and mallard-like hybrids lay more eggs and have higher survival rates than grey ducks and grey-like hybrids [Knox 1969, Heather et al. 2000, Williams and Basse 2006]. My study suggests a possible mating preference by females for mallard phenotype males, and thus sexual selection, to the list of potential causes. Two or all three of these factors may also work in concert.

Mating preferences of hybrids of other species pairs

Unfortunately, studies on the mating preference of hybrids are rare. For example, an experiment testing for the preference of Pecos pupfish (*Cyprinodon pecosensis*) and Sheepshead minnow (*C. variegatus*) females towards males of both species and hybrids

never tested the preference of the hybrids themselves [Rosenfield and Kodric-Brown 2003]. One exception is the above quoted study on *Heliconius* butterflies, that found hybrids to prefer other hybrids [Mavarez et al. 2006]. A study on the hybrids between the swordtail *Xiphophorus helleri* and the swordless platyfish *X. maculates* found that hybrid females and backcrosses prefer sworded males. Over time, the separate sworded species *X. clemencia* seems to have arisen from such backcrosses [Meyer et al. 2006]. As this shows, the preferences of hybrids, as well as those of the parental species, can clearly be crucial in shaping hybrid populations.

Conclusion and future outlook

I found evidence for two trends in hybridising mallard and grey ducks on the South Island of New Zealand. A survey of pairs suggested assortativeness of mating within both parental species and within hybrids. Secondly there was a tendency for females to pair with males more mallard-like in phenotype than the females were themselves. Both together may indicate a future duck population in New Zealand that contains little or no pure grey duck at all, but a variable hybrid swarm developing towards a mallard-like phenotype.

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Chapter 4

What the genes can tell us:

hybridisation of introduced mallard with native grey duck in New Zealand

Abstract

Worldwide, human induced hybridisation is causing increasing concern as a conservation threat. Often, introduced species threaten native ones by widespread introgression of genes, potentially eradicating one or both of the participating populations and leaving behind a hybrid swarm. The mallard (*Anas platyrhynchos*) is one of the most frequently implicated species in hybridisation. In New Zealand, hybridisation of native grey ducks (*Anas superciliosa*) with introduced mallards has been observed over most of the country, and concern has been raised as to the status of the grey duck. In this study, eleven microsatellite loci were used to investigate the genetic structure of the New Zealand population of grey ducks and mallards. New Zealand caught individuals were compared to samples of mallards including domestic ducks, and to grey ducks. The vast majority of New Zealand individuals appeared to be hybrids, and no unambiguous mallards or grey ducks were found in the sample. None of the individuals tested had a higher than 80% probability of being pure grey ducks. A phenotypic hybrid score was compared to the hybrid probabilities estimated from the microsatellite data. The correlation was significant, with phenotype score accounting for about half of the variation in genotype. This suggests that while plumage is not a perfect measure of hybridisation, and should be used only as a general guide to classify individuals in regard to their hybrid status in this species pair, it is a valid method for use in population studies.

Introduction

Hybridisation is an increasingly recognised conservation problem worldwide [Rhymer and Simberloff 1996, Haig 1998, Mooney and Cleland 2001, Levin 2002]. The mallard (*Anas platyrhynchos*) is one of the most frequently implicated species, and causes problems by hybridising with many other species of the genus *Anas*, which it has recently been brought into contact with through human introductions or range expansions due to human habitat alterations [Browne et al. 1993, Drilling et al. 2002, Rhymer 2006].

In New Zealand, extensive hybridisation between the introduced mallard and the native grey duck (*A. superciliosa*) threatens the native species. After mallards were introduced in the 1930s and 1940s [Knox 1969, Heather et al. 2000] the species spread rapidly from 1950 onwards, and with it hybridisation between mallards and grey ducks. A report by the Department of Conservation using phenotypic characters of more than 2000 ducks collected in the 1998 hunting season showed that animals with hybrid or mallard characteristics dominated in all areas sampled (ranging from 74% on the West Coast to 99% in Southland) [Williams 1998].

Hybrid status in this species pair has usually been assessed by phenotype, either using a phenotypic hybrid score [Rhymer et al. 1994] or less formal criteria. However, phenotypic scores offer only a rough estimate of actual genotypic hybrid status for many species pairs [Wilson 1992, Allendorf et al. 2001, Bronson et al. 2003]. Both overestimates and underestimates of the true values may ensue [Bensch et al. 2002, Randi and Lucchini 2002, Thulin et al. 2006]. In fact, it has been noted that hybrids between mallards and grey ducks in captivity do not always display phenotypic characters intermediate between the parental types [Phillips 1921, Braithwaite and Miller 1975].

Comparison of genotype and phenotype of birds of known hybridisation status from a breeding program showed that phenotypic characters are unreliable indicators of hybrid status [Williams 1998]. In particular, mallards and mallard-like hybrids were difficult to tell apart, and a discriminatory function was unable to separate grey-like hybrids from grey ducks [Williams 1998]. In addition, the hybrid score commonly used [Rhymer et al. 1994] regards any dark colouration in the bill of an individual as a sign of admixture with grey ducks, while mallard females commonly display black colouration in their bills [Audubon 1967, Cramp and Simmons 1977, Marchant and Higgins 1990, Heather et al. 2000, Drilling et al. 2002], and some other characters, such as the strength of an eye stripe or leg colouration, might be subject to interpretation and be classified differently by different researchers (pers. obs.).

Molecular methods offer another, increasingly important, route to detect hybridisation and investigate introgression [Haig 1998]. Amongst the commonly used molecular methods are those using microsatellites. Microsatellites are short, non-coding sequences of DNA that are repeated multiple times. The exact number of repeats is highly variable, and thus allows differentiation between individuals and populations. The resulting difference in length of DNA fragments cut before and after the repeated sequence is the measured variable [Queller et al. 1993, Blouin et al. 1996, Jarne and Lagoda 1996]. Microsatellites can be used to verify suspected cases of hybridisation or introgression, to monitor how much gene flow occurs and which geographic populations engage in it, and investigate if it is selective – that is if some adaptive genetic traits may introgress due to positive selection [see e. g. Gow et al. 2006, Lancaster et al. 2007, Vigfúsdóttir et al. 2008, Tung et al. 2008]. With investigations of mitochondrial DNA it can be found out if

introgression is bi- or unidirectional [see e. g Salzburger et al. 2002]. On the level of the individual, hybrid specimens can also be identified and distinguished from 'pure' individuals of parental species [see e. g. Ciucci et al. 2003, Pierpaoli et al. 2003, Thulin et al. 2006]

To name just three examples in ducks alone, microsatellite analysis has been used to identify hybrids of white headed and ruddy ducks [(*Oxyura leucocephala x O. jamaicensis*); Mueños-Fuentes et al. 2006]; show introgression of mallard into eastern spotbills [(*A. (poecilorhyncha) zonorhyncha*); Kulikova et al. 2004], and to demonstrate that while museum specimens several decades old showed a clear genetic distinction between mallard and American black ducks (*A. rubripes*), this difference has declined notably in modern specimens, a result of hybridisation [Mank et al. 2004].

To date, genetic work published on the hybridisation of mallard and grey duck is limited. The first attempt used 39 allozyme loci, but the main result was a distinct lack of diversity and heterozygosity over all populations tested [Hitchmough et al. 1990]. Restriction fragment analysis of mitochondrial DNA showed bi-directional gene flow, with hybrids carrying mitochondrial genotypes of both species [Rhymer et al. 1994]. However, in the absence of further molecular data, it is unknown exactly how much hybridisation occurs, and what the extent of hybridisation is in the current New Zealand population.

Here, I used highly variable microsatellite markers to identify hybrids between these two species, examine the extent of hybridisation and determine how good a proxy phenotype is for determining the hybrid status of individuals in populations where hybridisation is occurring. I predicted that (1) most NZ individuals would be identified as

hybrids by the microsatellite method, particularly all those displaying hybrid characteristics, and that (2) phenotypic characters, recorded as a hybrid score, predict the hybrid status as determined by the molecular method.

Methods

Sampling

A total of 89 ducks were collected in New Zealand in 2006 and 2007. Thirty six live ducks were captured in February and March 2006 and January to March 2007, and 53 dead ducks were collected in hunting seasons (May to July) of both years. A full list of all individuals with data on capture location and phenotypic hybrid scores is provided in the Appendix. Ducks were caught in funnel traps on agricultural land in HariHari (43˚ 8' south, 170˚ 34' east), on the West Coast of the South Island of New Zealand. According to Williams [1998], as well as my own work (see Chapter 2), the West Coast is among the regions in New Zealand where there is still a reasonably high proportion of ducks with a grey duck phenotype. The traps were cages of plastic-coated wire mesh with 1 m^2 base area. A 40 cm long funnel of the same material, that was 40 cm wide at the outside tapering to 12 cm on the inside, extended from one side of the trap to the centre. The design was based on Bub [1978], but the use of flexible plastic coated wire, connected with cable ties, allowed traps to be collapsible for transport and storage. I set up seven traps at the edges of ponds and brooks frequented by ducks, and baited with poultry wheat and bread. Traps were controlled, and bait replenished at least twice a day, in the early morning and just before sundown. If necessary, for example when livestock was moved onto a field, the traps were moved to other trapping locations. Any non-target

birds caught, such as pukeko (*Porphyrio porphyrio*), sparrows (*Passer domesticus*), thrushes (*Turdus philomenos*) and others, were released immediately. Ducks captured were transported in cloth bags to the Charles Foweraker field station of the University of Canterbury in HariHari for blood to be taken by venipuncture at the wing. To prevent the spread of infections these bags were washed between uses. Dead ducks were obtained from hunters, usually within hours of death. Forty-eight of the ducks were collected in HariHari, and five in Haast (43˚ 52’ south, 169˚ 2’ east). The blood of dead ducks was not always fresh enough to sample, thus in these cases a toe web clipping was taken instead. Samples were stored in lysis buffer (100mM NaCl, 50mM Tris pH8, 1% SDS, 10mM EDTA). DNA was extracted by chloroform phenol extraction [modified from Sambrook et al. 1989], and DNA was resuspended in 1 x TE buffer. Extracted DNA was obtained from 36 Australian grey ducks (*Anas superciliosa*), 12 North American mallards and 10 domestic ducks (both *Anas platyrhynchos*). Domestic ducks were included as populations of domestic mallard will have reached New Zealand in significant numbers before wild mallards, and many New Zealand populations contain individuals of obvious domestic ancestry.

Phenotypic analysis

A phenotypic hybrid score following Rhymer et al. 1994 (see appendix) was also taken from each duck. Briefly, the colouration of bill, head, speculum and legs was considered, and scores assigned to each individual for each of these characters. Total scores can vary between 2 for a pure grey duck phenotype to 25 for a mallard drake, and 24 for a mallard female.

Genotyping

Eighteen microsatellite loci developed in the mallard [Maak et al. 2003] were trialled on samples of hybrid ducks from New Zealand, and those that worked consistently were selected for further analysis. All samples were genotyped for eleven microsatellite loci as described in Maak et al. [2003]. The loci selected were: APH12, APH13, APH15, APH16, APH17, APH19, APH20, APH21, APH23, APH24, and APH25 (EMBL/GenBank accession numbers AJ515888, AJ515889, AJ515890, AJ515891, AJ515892, AJ515894, AJ515895, AJ515896, AJ515897, AJ515898, AJ515900) [Maak et al. 2003] (see Table 1). In addition to the published sequences, each forward primer had an additional M13 sequence tag added to the 5' end to enable flexible and cost effective multiplex sets to be established [Oetting et al. 1995, Schuelke 2000].

PCRs were set up as 10 µl reactions containing 1x buffer (Bioline), 0.1 mM reverse primer, 0.5 mM M13 forward primer, 0.1 mM fluorescent tag, 1.5 mM $MgCl_2$ (Bioline), 0.2 mM dNTPs (Bioline), 1 unit Taq (Bioline) and 0.5 µl DNA (~0.05-0.1 ng/µl). Thermocycling started with denaturing at 95˚C for 60 sec, followed by 30 cycles of 94˚C for 40 sec, Ta˚C for 30 sec, and 72˚C for 30 sec, followed by a further 10 cycles of 94˚C for 40 sec, 53˚C for 30 sec, and 72˚C for 30 sec. Annealing temperatures for each primer pair were as previously published [Maak et al. 2003] (Table 1). Negative controls were included in each run. For each reaction 4 µl of PCR product was run on a 2% agarose gel made up in 1xTBE, which was stained with ethidium bromide and visualised over a UV illuminator to determine if amplifications were successful prior to genotyping.

All genotyping was performed on an ABI3100 Genetic Analyser (Applied Biosystems Inc) by the University of Canterbury sequencing service. Up to four PCR

products, with different fluorescent tags (FAM, NED, VIC, PET; Applied Biosystems), were used in the same genotyping reaction. Genotyping reactions consisted of 2 µl mixed PCR products, 10 µl formamide, 0.3 µl Gene Scan 500LIZ size standard, and 8 µl H_2O, and these were denatured at 95˚C for 5 min prior to electrophoresis.

Scoring and binning of genotyping data

Microsatellite allele lengths were determined by comparison to an internal size standard using Gene Marker 1.71 (Applied Biosystems). Flexibin [Amos et al. 2007] was used to automatically bin alleles into their likely size classes. This program automatically converts raw allele lengths into allele classes, allowing for imperfect colinearity between measured and actual fragment length. Flexibin has some advantages over classical binning by eye, as it uses a statistically robust and reproducible approach to establish allele size. This reduces the incidence and thus effect of spurious allele sizing in the data [Amos et al. 2007]. Nonetheless the software is not perfect and the automatically determined sizes were corrected by hand in the few cases where the graphs provided by the program suggested that binning by the algorithm was likely incorrect [Amos et al. 2007]. Graphs of the binning classes and tables of their sizes and frequencies are presented in the Appendix, together with notes on any alterations performed. These size classes were used for further analysis. Results found using this method were similar to those found binning the data by eye and then rounding to the nearest base.

Table 1. Eleven microsatellite loci used in this study, with the allele sizes reported in Maak et al. [2003] and those found here, with the numbers found here across all three populations. Note that my allele sizes are increased by 20bp over those of Maak et al. due to the addition of the M13 sequence on the forward primer.

Locus	**Primer Sequence (F:5'-3' and R:5'-3')**	**T_a (˚C)**	**Maak et al: allele size (bp)**	**Allele size (bp)**	**Allele number**
APH12	F: TTA GTA GCA TGT CAG GTT TAT T R: GCT TGT AGA CTT CAG AGT TC	52	165	156-179	12
APH13	F: CAA CGA GTG ACA ATG ATA AAA R: CAA TGA TCT CAC TCC CAA TAG	52	179	187-212	14
APH15	F: TGA ATA TGC GTG GCT GAA R: CAG TGA GGA ATG TGT TTG AGT T	60	179	190-199	3
APH16	F: CCT TCT GAA CCT TCG TAG R: AAA TAT AGA CTT TTG TCC TGA A	52	146	163-167	3
APH17	F: GGA CAT TTT CAA CCA TAA ACT C R: CAT CCA TGA CAG ACA GAA GA	60	222	226-248	10
APH19	F: CAT GGA GCA AGC AAT CGT CTG R: ACC ACG TCC ATC CTG AAG AAA	54	166	181-189	5
APH20	F: ACC AGC CTA GCA AGC ACT GT R: GAG GCT TTA GGA GAG ATT GAA AAA	58	150	158-172	8
APH21	F: CTT AAA GCA AAG CGC ACG TC R: AGA TGC CCA AAG TCT GTG CT	59	137	148-172	12
APH23	F: TCC TCT GCT CTA GTT GTG ATG G R: CCT CAG CAG TCT TCC TCA GTG	58	205	210-244	14
APH24	F: TCA ACC AGT GGT CAG AGA AAA A R: AGG TCA GCC CCC ATT TTA GT	58	147	152-176	11
APH25	F: CCG TCA GAC TGT AGG GAA GG R: AAA GCT CCA CAG AGG CAA AG	58	167	182-188	5

Analysis of genotypic data

Deviations from Hardy Weinberg equilibrium were tested for using Genepop 3.4 [Raymond and Rousset 1995]. Overall, there was a significant departure from the level of heterozygosity expected under Hardy Weinberg equilibria and that expected in my data. In the domestic ducks, 8 of 11 loci conformed with the Hardy Weinberg equilibrium, while in the mallards 4 of 11 loci were in Hardy Weinberg equilibrium and in grey ducks 3 of 10. 5 of 11 loci met Hardy Weinberg expectations in the New Zealand ducks.

Table 2. Probability that each locus is in Hardy Weinberg equilibrium in each population. Significant deviations at p= 0.05 are marked with *.

Locus	**Grey ducks**	**Domestic ducks**	**Mallards**	**New Zealand population**
APH12	0.0000 *	1.0000	0.0037 *	0.0000 *
APH13	0.0155 *	0.0488 *	0.0000 *	0.0000 *
APH15	0.0612	0.0568	0.2308	0.0947
APH16	0.5922	1.0000	1.0000	0.0911
APH17	0.0000 *	0.0486 *	0.0019 *	0.0000 *
APH19	0.0001 *	0.0752	0.4577	0.0000 *
APH20	0.0000 *	0.0664	0.0211 *	0.0000 *
APH21	0.0219 *	1.0000	0.0000 *	0.0000 *
APH23	0.2498	1.0000	0.3230	0.0613
APH24	0.0117 *	0.0224 *	0.0167 *	0.2028
APH25	na	0.4253	0.0013 *	0.4576

The program Structure 2.2.3 [Pritchard et al. 2000] was used for Bayesian cluster analysis of the microsatellite data. Several runs without use of *a priori* information on the

origin of individuals confirmed the presence of two distinct populations conforming to the two species. For the main analysis, individuals of non-hybridising populations from both species were specified as known populations 1 and 2, and the New Zealand ducks were assigned by the program a probability (Q) of belonging to either population, with the following settings: Gensback 2, migprior 0.005, and independent allele frequency. The burn-in and Monte Carlo Markov Chain run lengths were of 200,000 each. The decision to use two populations was verified by testing the fit of the data to population numbers (K) of two through four. Alpha, which describes the rate of convergence of the Markov chain, was observed to converge to ensure that burn-in and run times were sufficient to avoid settling on sub-optimal solutions [Pritchard et al. 2000]. The program was run three times with these settings, and differences in the results between the runs were small providing confidence in the robustness of our estimates. Q, the estimated membership probability of an individual for each cluster, was used as a measure of hybrid ancestry.

Regression of the genetic data on phenotypic scores was performed in SPSS (17.0.0).

Results

Genotypic data

All eleven loci were polymorphic, having between 3 and 14 alleles, with an average of 8.8 alleles. Alleles differed between the two species and the New Zealand population, with up to 7 alleles per locus (average 1.2 alleles) occurring in mallards not being found in grey ducks, and up to 6 (average 2.4 alleles) of those found in grey ducks not found in

this sample of mallards (Table 3). On average, the New Zealand duck population had 5.6 alleles per locus.

Table 3: Allele numbers, for each locus and population, as found in Maak et al. [2003], and in this study. {Numbers, n} are sample sizes, and (numbers) are the number of alleles of grey duck or mallard not found in the other species in this study.

Locus	**Maak et al.: Peking Duck {n=40}**	**Maak et al.: Mallard {n=5}**	**Mallard {n=12} and domestic {n=10}**	**Grey duck {n=36}**	**New Zealand population {n=89}**
APH12	2	7	7 (0)	8 (1)	10
APH13	6	6	5 (1)	8 (4)	13
APH15	3	2	3 (0)	3 (0)	3
APH16	4	3	2 (0)	3 (1)	3
APH17	2	8	6 (2)	6 (2)	8
APH19	2	3	4 (1)	4 (1)	5
APH20	3	4	5 (0)	7 (2)	7
APH21	4	6	4 (1)	10 (6)	9
APH23	5	2	5 (0)	10 (5)	13
APH24	5	2	8 (7)	5 (4)	7
APH25	3	3	4 (1)	2 (0)	4
Average	**3.5**	**4.2**	**4.8 (1.2)**	**6.0 (2.4)**	**5.6**

All individuals of known origin, both mallards and Australian grey ducks, were correctly assigned to their species cluster with > 0.9 probability. The New Zealand duck population sampled appears to be of mixed ancestry. Overall, an estimated 58.7% of

ancestry stems from mallard and domestic ducks, and the remaining 41.3% from grey ducks. Probabilities ranged from 0.057 to 0.943. Not one unambiguously pure individual of either mallard or grey duck (> 0.99 or < 0.01 probability) was found in this sample of 89 ducks, but 6 individuals had > 0.9 probability, so might be mallards rather than hybrids, and 3 had a probability of < 0.1, so might have been pure grey ducks [Vähä and Primmer 2006].

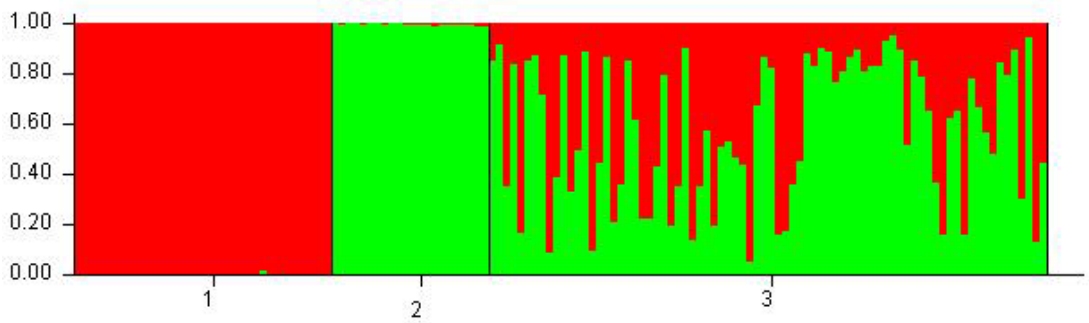

Figure 1. Barplot showing the probability of individual ducks to be of one of two populations, namely grey duck (1), or mallards (2), as calculated by Structure (Pritchard et al. 2000) using data from 11 microsatellite loci. None of the ducks sampled in New Zealand (3) have a chance > 0.96 of being mallard, or > 0.94 of being a grey duck.

Phenotypic data

In the 89 ducks, phenotypic scores ranged from 3 to 25. Therefore, no ducks of an unambiguous grey phenotype were encountered, and only two individuals, one male and one female, were of pure mallard phenotype. All other individuals were of hybrid appearance of varying degrees.

Comparison of genotypic and phenotypic data

There was a significant correlation between the phenotypic score, and the genotypic probabilities estimated ($p < 0.001$) (Figure 2), but phenotypic score only explains about 53% of the variation observed in the genotypic estimate ($r^2 = 0.533$). Individuals with genotypic profiles that are within a probability of > 0.9 can show what appear to be clear signs of hybridisation in their plumage, and individuals with quite high or low phenotypic scores, that appear similar to pure mallards or grey ducks, can show clear signs of admixture in their genomes.

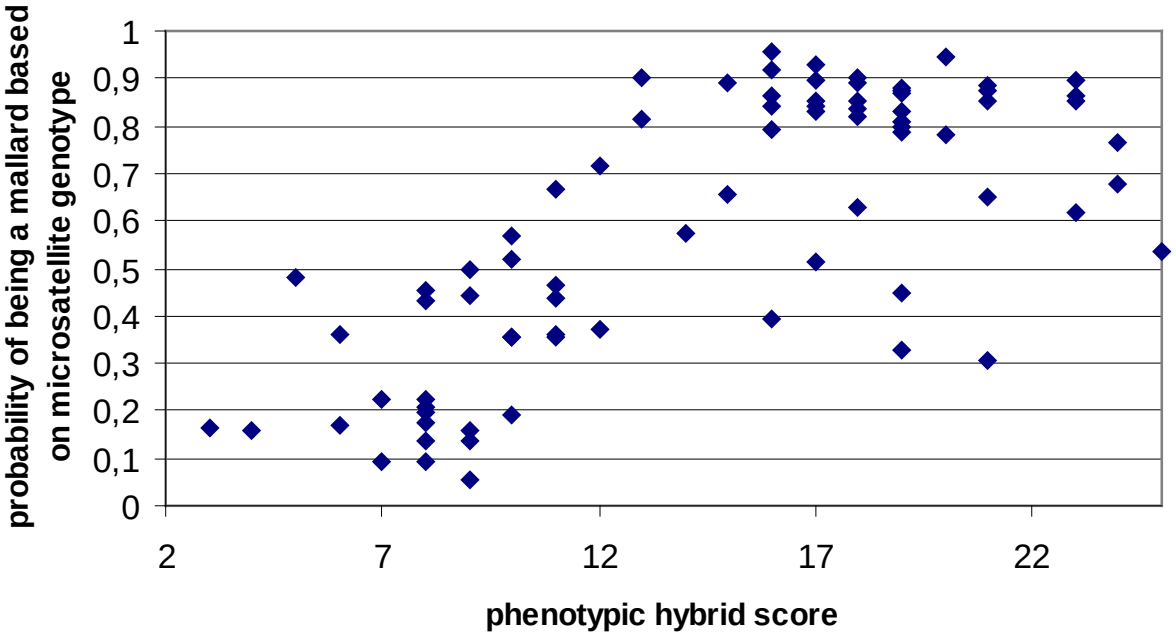

Figure 2. The probability of individual New Zealand ducks to be pure mallards as estimated by Structure (Pritchard et al. 2000), plotted against phenotypic hybrid scores (ranging from 2 for the pure grey duck phenotype to 25 for the pure mallard phenotype). The correlation is significant, but phenotype fails to explain about 47% of variation in genotype.

Discussion

Summary of results

Eleven microsatellite markers were selected to assess hybridisation in New Zealand between native grey ducks, and introduced mallards. Bayesian analysis using Structure strongly suggests that hybridisation is common in the New Zealand population tested. In fact, no unambiguously pure specimens of either species were contained within the sample. Overall, about 60% of the genetic material within the population was estimated to stem from mallards, with the remainder contributed by grey ducks. Phenotypic hybrid scores of individuals were found to only be a rough guide to the genetically determined hybrid probabilities.

The sample included only two individuals – a male and a female – with a pure phenotype. Both were of mallard phenotype, but neither scored a high probability of being mallards using microsatellite data (0.535 for the male, 0.767 for the female). These probabilities were comparable, and even lower, than those of other individuals whose plumage indicates that they were highly likely to be hybrids. Both are likely to have been cryptic hybrids.

Limitations in the interpretation of the microsatellite markers

For reliable analysis of the hybrid status of individuals, more than the eleven markers used in this study would be desirable [Vähä and Primmer 2006]. For this reason, no attempts were made to distinguish possible first generation hybrids or backcrosses from latter generation hybrids, although there are analytical tools that allow such analyses to be undertaken, as for example NewHybrids [Anderson and Thompson 2002]. However, the

highly polymorphic nature of the microsatellite loci used here, and the fact that a quarter of mallard alleles and a third of grey duck alleles were diagnostic of that species, suggests that the results should be sufficient to give an accurate idea of the hybridicity of the total sample population.

All loci scored were dinucleotide repeat loci, with the exception of APH12 which followed a more complex repeat pattern $(GAAA)_4A_2(GAAA)_2$ [Maak et al. 2003], and thus the expected distribution of alleles is generally based on double repeats. However, as is often the case in scoring microsatellite loci, some alleles were scored as single base shifts rather than as dinucleotide shifts. Where these are common they may well be genuine, but where rare most likely reflect a gel shift, perhaps due to high salt concentration, in the running of that sample. Without extensive repeat analysis and ideally direct sequencing of alleles it is not easy to determine the exact size of a given allele, thus the conservative approach is to overestimate similarity by grouping alleles within a base pair of each other in size into allelic ‘bins’. This analysis can be done by eye, but automated approaches have the advantage of reproducibility [Amos et al. 2007]. Thus for my analysis I chose to employ automated binning using the Flexibin software [Amos et al. 2007]. This reduced the number of alleles in the analysis, and reduced the effect of single repeat alleles on the downstream analysis. However, the graphical and tabular output of Flexibin for some loci (especially APH12) suggests that some single base alleles do exist in my data (see Appendix). For the purpose of downstream analysis, I assume that using the automatically binned alleles reduces the error, and delivers more reliable results. However, results from an analysis using the data as binned by eye

undertaken earlier, without the use of Flexibin, were similar if not identical (data not shown), and the main conclusions were all the same between both methods.

An error estimate calculated from repeated genotyping of a part of the sample would have helped to further assess the reliability of the data presented here [Hoffman and Amos 2005], but such data are unavailable. However, all genotyping was performed on the same sequencer within a short period of time, thus potential genotyping errors that might be induced from changes in sequencer setting, changes in reagents, and variability among machines is likely to be small. For each locus, all samples of the mallard, domestic duck and grey duck were carried out on the same plates, and all New Zealand ducks were also run on the same plate, thus there is likely to be little variability among runs. The only exceptions to this were a few datapoints (n=5 to n=14) for some loci (n=6) for which results were not satisfactory in the first attempt, and checking these reruns by eye shows they do not differ systematically from those of other ducks in the first run.

Deviations from Hardy Weinberg equilibrium

The data in many loci and populations did not fit the expectations of Hardy Weinberg equilibrium. This can be a sign of scoring errors – if, for example, heterozygous loci are mistakenly scored as homozygous. While that possibility cannot be discounted completely, several facts indicate that the populations might instead simply show allele frequencies out of Hardy Weinberg equilibrium. First, Maak et al. [2003] found the same deviation in 6 of the 11 loci used here in their original report of these loci. Second, a pattern over multiple loci, rather than just one or two, is likely to reflect underlying demographic patterns, such as small population sizes, inbreeding, or population

subdivisions. The samples used from both mallards and grey ducks were collected over large geographic areas in their countries of origin, and therefore panmixis, a core assumption of Hardy Weinberg, was not expected. The New Zealand population might, despite the widespread hybridisation, still show signs of the two species because mating is non-random (see Chapter 3), which is an obvious departure from the expectation of random mating under Hardy Weinberg. It is thus interesting to note that the domestic ducks, for which a more homogenous population may be assumed, had a higher rate of fitting Hardy Weinberg expectations than the other populations.

Limitations caused by the sample ranges

While the hybridisation between the grey duck and mallard in New Zealand has long since been noted and suspected to be problematic, the genetic evidence presented here is a further indicator that the situation has gone beyond one of limited hybridisation and introgression. The sample used here was too limited, both in number and geographical sampling area, to allow a firm conclusion about the total New Zealand duck population, but the two species might well be in the process of merging into one continuous breeding population, at least locally.

Larger samples of birds, including mallards from Europe, and museum specimens of grey duck from New Zealand pre-dating the hybridisation, would increase the sensitivity of the analysis. New Zealand grey ducks have previously been shown to possess some mitochondrial genotypes very different from those of Australian grey ducks, in addition to more similar genotypes [Rhymer et al. 2004]. It is unknown how much genetic diversity there is within the nuclear DNA of the species in its total range.

A geographically wider sample of contemporary New Zealand ducks might allow a more general assessment of the situation. However, the West Coast, at which all New Zealand birds were sampled, is considered one of the areas in New Zealand with the lowest level of hybridisation between the species [Williams 1998, see Chapter 2]. Despite the limited sampling range and sample size, the results presented here suggest that the native grey duck might be imperilled as a species by the hybridisation that has already occurred with mallards.

Suggested further work

The microsatellite loci discussed here offer, for the first time, a tool to thoroughly examine hybridisation between these two species without relying on phenotypic characters. It is strongly recommended to repeat a similar analysis in Australia, and other locations where mallards have been introduced into the range of the grey duck. This would allow a more accurate picture of how advanced and widespread hybridisation is, and might increase chances of successful management. This is becoming critical not for isolated populations, but for the survival of the grey duck as a distinct species. This approach could also be used to identify any individuals of the native species persisting within New Zealand, or elsewhere. However, for a reliable analysis at the level of individuals, additional markers are desirable.

Additionally, these nuclear markers could also be used to investigate preserved samples of the Mariana's mallard (*Anas oustaleti*), an extinct population on the Mariana's Islands, that has been hypothesized to have been a hybrid population between mallards

and grey ducks [Yamashina 1948]. The debate as to whether the Mariana's mallard was a hybrid population or not might thus be settled.

Reliability of phenotypic scores in hybrid identification

Apart from elucidating the situation of the grey duck and mallard hybridisation in New Zealand, this study provides further evidence that phenotype is only a rough indicator for hybrid status. This has previously emerged as a problem in various studies of other species, for example in black-capped and Carolina chickadees (*Poecile atricapilla* x *P. carolinensis*) [Bronson et al. 2003] or in clams (*Corbicula spp.*) [Pfenninger et al. 2002]. In the latter case, hybrid phenotypes were much rarer than hybrid genotypes, possibly due to a fitness disadvantage to intermediate types. In the mallard and grey duck, it appears that the correlation between genotype and phenotype is weak, but existent. While phenotypic scores can be useful for assessing, for example, changes in the proportion of a population made up of hybrids over time or between locations, phenotypic hybrid scores have limits and cannot always reliably be used to assess hybrid status of individuals.

In the case of grey duck and mallard, hybrid identification by phenotype should not be used to identify pure individuals, as cryptic hybrids (n=2 of 78 total, n=2 of 2 individuals of pure phenotype) have been found to occur within the sample analysed here. The phenotype of an individual cannot be used to identify non-hybridised individuals, nor can it be used to determine hybrid status or genotypic status of any individual. At the same time, a general correlation between phenotypic and genotypic hybrid scores indicates that phenotypic scores can be used as a measure on a population scale (as in

Chapters 2 and 3). The deviations of phenotypic measure from the genotype can in some cases over- and in other underestimate the proportion of mallard genome in the hybrids. Such random, non-systematic deviations will in a large enough sample not pose a large problem for a statistical analyses, as statistical analyses are after all specifically designed to deal with such random deviations. Keeping this in mind, it is possible and justifiable to use hybrid scores when taking large samples and to assess entire populations. They should never be used uncritically in this species pair however to assess hybrid status of individuals. While caution should thus be used, phenotypic scores can allow a fast method to assess a large number of individuals, without capture or samples being collected.

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Chapter 5

How are hybrids faring?

Condition-related fitness of grey ducks, mallards and their hybrids

Abstract

Condition-related components of fitness were compared between grey ducks (*Anas superciliosa*), mallards (*A. platyrhynchos*), and their hybrids in New Zealand. Condition was measured by estimating ectoparasite loads and endoparasite presence, relative white blood cell counts, and by conducting an immunochallenge experiment with a foreign protein. Few of the 89 ducks sampled over two years were classified as pure parental species, but hybrids varied in the degree to which they resembled one or the other parental species, and I used both phenotypic and genotypic scores to measure degree of hybridisation and its influence on condition. Only weak evidence was found for any differences between mallard-like and grey-like individuals in any of the condition-related measures. The results were similar whether I used either phenotype or genotype as determined by microsatellite analysis. It appears that in the current hybrid swarm between the two species, there is no large advantage or disadvantage in condition to individuals closer to either parental type, but weak evidence suggests that should an advantage exist, it is for individuals of a mallard-like type.

Introduction

Knowledge about the relative fitness of hybrids and their parental species is helpful for understanding and predicting how a hybridising population develops and persists. The classic view that hybrids are invariably less fit than either of the parental species can no longer be upheld [for a summary of this classic view see for example Soulé 1986, or most other standard textbooks]. In plants it has long been accepted that hybrids can be either fitter, equally fit or less fit than the parental types, but in animals this view of variable outcomes has been a more recent development [Arnold et al. 2001; Burke and Arnold 2001].

Studies of the parasite loads of hybrids provide a good example of the range of possible fitness outcomes of hybrids. In some cases, parasites may be more prevalent on hybrids than on either parental species [Dupont and Crivelli 1988]; parasite loads are higher in *Daphnia* hybrids and may even limit hybrids that are otherwise more fit than their parental types [Wolinska et al. 2004]. On the other hand, hybrids may be more resistant to parasites than either parental species. For example, hybrids of the frogs *Xenopus laevis* and *X. muelleri* are more resistant to monogenean polystome parasites than the parental species [Jackson and Tinsley 2003]. Parasites may also occur on hybrids with prevalences proportional to the genome shared with either parental species [le Brun et al. 1992].

Hybrid fitness relative to their parental species can also depend on the environment. Three-spined stickleback (*Gasterosteus aculeatus* complex) hybrids show high fitness in the laboratory, but in the wild are inferior to each parental type in their respective environments [Hatfield and Schluter 1999]. In marine *Littorina* snails, hybrids are also

less fit in the environment of either parental species; however, in an intermediate zone between the parental environments all three genotypes – both parentals and hybrid – are of equal fitness [Rolán-Alvarez et al. 1997]. Fitness may also vary temporally. Hybrids between the Darwin's finches *Geospiza fortis* and *G. scandens* have lower fitness in most years, but in especially dry years the hybrids are exceptionally fit since the intermediate beak size and shape of hybrids allows them to use all of the few seeds available [Grant et al. 2003, Grant et al. 2004]. In one case, hybrid fitness has even been shown to be age-dependent, with young *Drosophila pseudoobscura x D. persimilis* hybrids being less fecund, but old individuals more fecund than either parental species [Promislow et al. 2001].

Because fitness varies between species pairs as well as with location and conditions, it has not been possible to predict the fitness of any given hybridising population. Instead, each situation appears unique and studies are necessary to determine the outcome. To complicate matters, relative fitness of a hybrid in one measure does not always predict fitness in other measures. For example, hybrids between Townsend's warblers (*Dendroica townsendi*) and hermit warblers (*D. occidentalis*) are intermediate to parental species in regard to finding territories and mates, but their lifespan is shorter [Pearson 2000]. Fledgling success of hybrids between collared (*Ficedula albicollis*) and pied flycatchers (*F. hypoleuca*) is the same as that of the parental species. However, less hybrid young breed [Atalato et al. 1982], and all female hybrids are sterile [Gelter et al. 1992]. It has therefore been suggested that studies into hybrid fitness should include multiple measures [Arnold and Hodges 1995].

Measuring fitness directly is difficult, and instead fitness components such as breeding success, survival, body condition and health-related measures are frequently measured to allow an estimate. Within each of these categories, a large range of methods are available, and adequate ones need to be chosen for each situation. Health-related measures for example include estimates of body condition, parasite counts, fluctuating asymmetry, and measures of immunocompetence, such as relative white blood cell counts or reaction to immunochallenge with a foreign protein [Norris and Evans 2000, Lee 2006]. Immunocompetence is considered as the ability of an individual's immune system to deal with challenges such as bacteria or parasites. It consists of various parts, such as the variety and number of antibodies produced, or cell-mediated immunity, and it is determined both by genetic and acquired factors, as well as the current condition of an individual [Goldsby et al. 1999, Eales 2003]. It can therefore be difficult to measure and the measures in turn can be difficult to interpret (see discussion). Nevertheless, measure of immunity can provide insight into the fitness of an individual and may help elucidate whether differences in fitness between parental species and hybrids plays a role in the progress of hybridisation.

In species were hybrids show increased fitness, first generation hybrids tend to be more fit than later hybrid or backcross classes, since they do not suffer from the break-up of co-adapted gene complexes, and often display increased fitness (called hybrid vigour or heterosis), while later generations may suffer decreased fitness (called hybrid breakdown or outbreeding depression) [Edmands 1999, Marr et al. 2002]. To assess the long-term effects of hybridisation, it is therefore necessary to understand the effects of the hybridisation on the fitness of later generation hybrids.

Hybrid swarms offer an ideal study system for more detailed analysis of the interrelation of hybrid status and fitness, since they contain a whole range of mosaic genomes, including later generation hybrids. Investigations in hybrid swarms can give an insight into how partial genomes work together, into the extent to which the break-up of co-adapted gene complexes is a lasting problem, and into the role of selection on hybrids. Despite the potential usefulness of comparing fitness among individuals in a hybrid swarm, such an analysis simultaneously investigating a number of fitness measures has not yet been attempted, and few studies have looked even at a single measure. An attempt to link hybrid status and growth rate in extensively hybridising coastal steelhead trout and coastal cutthroat trout in North America yielded inconclusive results [*Oncorhynchus mykiss irideus* x *O. clarki clarki*; Ostenberg et al. 2004]. A study on Pecos pupfish (*Cyprinodon pecosensis*) and sheepshead minnows (*C. variegatus*) found Pecos pupfish to be less enduring swimmers than either sheepshead minnows or their hybrids, and that first generation hybrids and sheepshead minnows grow faster than Pecos pupfish [Rosenfield et al. 2004]. Better insight into the fitness of hybrids is needed, and possible only via studies using multiple measures.

The large hybrid swarm between grey ducks (*Anas superciliosa*) and mallards (*A. platyrhynchos*) in New Zealand offers an ideal opportunity to examine hybrid fitness, not just in the first or second generation, but at much later stages. Using a variety of measures of condition such as immunocompetence and parasite loads, I compared the health of individuals that differed in their degree of hybridisation. While it is difficult to predict the fitness of hybrids, in this case I expected hybrids to be comparatively fit in relation to the parental species, particularly so in comparison to the grey duck. This prediction was

based on the observation that hybrids are widespread, common, and increasing in proportion relative especially to the parental grey duck (Chapter 2). These data should help understand the dynamics within the population, and may help predict the future of the grey duck population in New Zealand.

Methods

Capture of live individuals

Thirty-six ducks were live-captured in February and March 2006 and January to March 2007, and 53 dead ducks were collected in the hunting seasons (May to July) of 2006 and 2007. Ducks were live-captured in funnel traps on agricultural land in HariHari (43˚ 8’ south, 170˚ 34’ east), on the West Coast of the South Island of New Zealand. According to Williams [1998], as well as my own work (see Chapter 2), the West Coast is among the regions in New Zealand where there is still a reasonably high proportion of ducks with a grey duck phenotype.

Traps were made of plastic-coated wire mesh with a base area of 1 m^2. A 40 cm long funnel of the same material, shaped to be a semicircle of 40 cm width at the outside and tapering to 12 cm wide on the inside, extended from one side of the trap to the centre. The design was based Bub on [1978], but the use of flexible plastic coated wire, connected with cable ties, allowed traps to be collapsible for transport and storage. I set up 7 traps at the edges of ponds and brooks frequented by ducks. Each trap was baited with poultry wheat and bread inside and outside of the trap. Bait was replenished at least twice every day, in the early morning and just before sundown. If necessary, for example when livestock was moved onto a field, the traps were moved to other trapping locations. Any non-target birds caught, such as pukeko (*Porphyrio porphyrio*), sparrows (*Passer*

domesticus), thrushes (*Turdus philomenos*) and others, were released immediately. Ducks captured were transported in cloth bags to the Charles Foweraker field station of the University of Canterbury in HariHari for measures to be taken. To prevent the spread of infections, or cross-contamination in the counts of ectoparasites, these bags were washed between uses.

Physical records

All ducks were banded. Three photographs were taken of each individual – one of the entire duck from the side, one of the head, and one of the speculum in the wing. Weight and the lengths of tarsus, wing, skull and bill were recorded to the nearest millimetre, in order to calculate relative body mass. A hybrid score following Rhymer et al. [1994] (see Appendix) was taken.

Blood samples and slides

A blood sample was taken by venipuncture at the wing and stored in lysis buffer (100mM NaCl, 50mM Tris ph8, 1% SDS, 10mM EDTA) for DNA analysis. Additionally, two blood smear slides were prepared for each individual from fresh blood. A drop of blood was transferred to the slide, and smeared along the slide surface with the edge of another slide, with the aim of obtaining in a single cell layer. The slides were later stained with May-Grunewald and Giemsa stains for relative counts of heterophils and lymphocytes under the microscope. A total of at least 200 white blood cells was counted for each individual bird. Eleven of the 78 individuals could not be scored as

quality of the smear was not sufficient. These were all individuals collected after death, so it is possible that natural decomposition processes were responsible.

Parasites

Ectoparasites were assessed by dust-ruffling the plumage of ducks with flea powder (19.5g/kg permethrin, VitaPet) for five minutes per bird. The duck was held over a large paper sheet in a location free of any air movements, and the powder worked into the feathers for the full five minutes, attending to all body parts equally, excepting the face. All parasites, feathers, and skin flakes removed were collected on paper sheets and put into plastic bags for sorting and analysis in the laboratory. The parasites were identified to the level of genus. To test for endoparasites, a faecal samples was taken from live individuals in 2007 (the samples from 2006 were destroyed by technical problems), and send to Gribbles Veterinary Pathology (Christchurch) for analysis. Because the populations of parasites in fecal samples tend to fluctuate depending on the parasites' life cycle, a single sample does not always allow a reliable measure of the number of parasites present. As multiple measures could not be taken in the wild animals, no qualitative analysis was attempted; instead only the presence or absence of internal paratsites was scored. False negatives are nonetheless possible – some individuals scored as free of endoparasites might have been infected.

PHA immunochallenge

Ducks were held overnight in purpose-built holding cages of 1 m^2 area, similar in design to the traps but without a funnel. These were placed at a quiet location behind the

field station in HariHari and were not visible from the road. Poultry wheat and water were available at all times. To prevent the spread of infections, the holding cages were moved to fresh areas of grass between occupants.

PHA (PHA-P phytohaemagglutinin, Sigma) was used to assess non-specific immune response. A 1 cm^2 area of the wing web of each wing was cleared of feathers using a pair of blunt scissors and the thickness of the wing web was measured using digital calipers. Three measurements were taken, and the average used as final measurement. One of the wings was then injected with 100 micrograms of PHA in 100 microlitres phosphate buffered saline solution (Sigma) [following recommendation for pintail ducks *Anas acuta*, Smits et al. 1999] at approximately 2200 h New Zealand Standard Time. Both wings were measured again ten hours later to assess the resulting swelling. Following these measurements the ducks were released.

Dead specimens

Dead ducks were obtained from hunters on the day of shooting, usually within a few hours of death. Fourty-eight of the ducks were collected in HariHari, and 5 in Haast (43˚ 52’ south, 169˚ 2’ east). Measures were taken as described above, with the following differences. Blood was not always fresh enough to make blood slides or to take blood samples. A toe web clipping was taken instead for DNA analysis when applicable. Dust ruffling was not attempted, since hunters stored all shot birds in one bag and cross contamination was likely. A PHA analysis is also not possible in dead animals.

Statistical analyses

Measures were compared both to the phenotypic scores of each individual and a genetic score established using ten microsatellite loci (see chapter 5). Phenotypic scores are not always an accurate measure of the hybrid status of an individual (see previous chapter). However, I present analyses of both phenotypic and genotypic data here for completeness, and because phenotype could, in some cases, be suspected to correlate with fitness independent of underlying genotype. However, tests conducted using genotypic scores are likely to be more accurate than those conducted using phenotypic scores. ANOVAs were used when comparing one categorical variable with a continuous one, regressions when comparing two continuous variables, and General Linear Models when a third variable had to be controlled (the swelling of the control wing in PHA analysis). Statistics were performed in Minitab (15.1.0.0) or SPSS (17.0.0).

Results

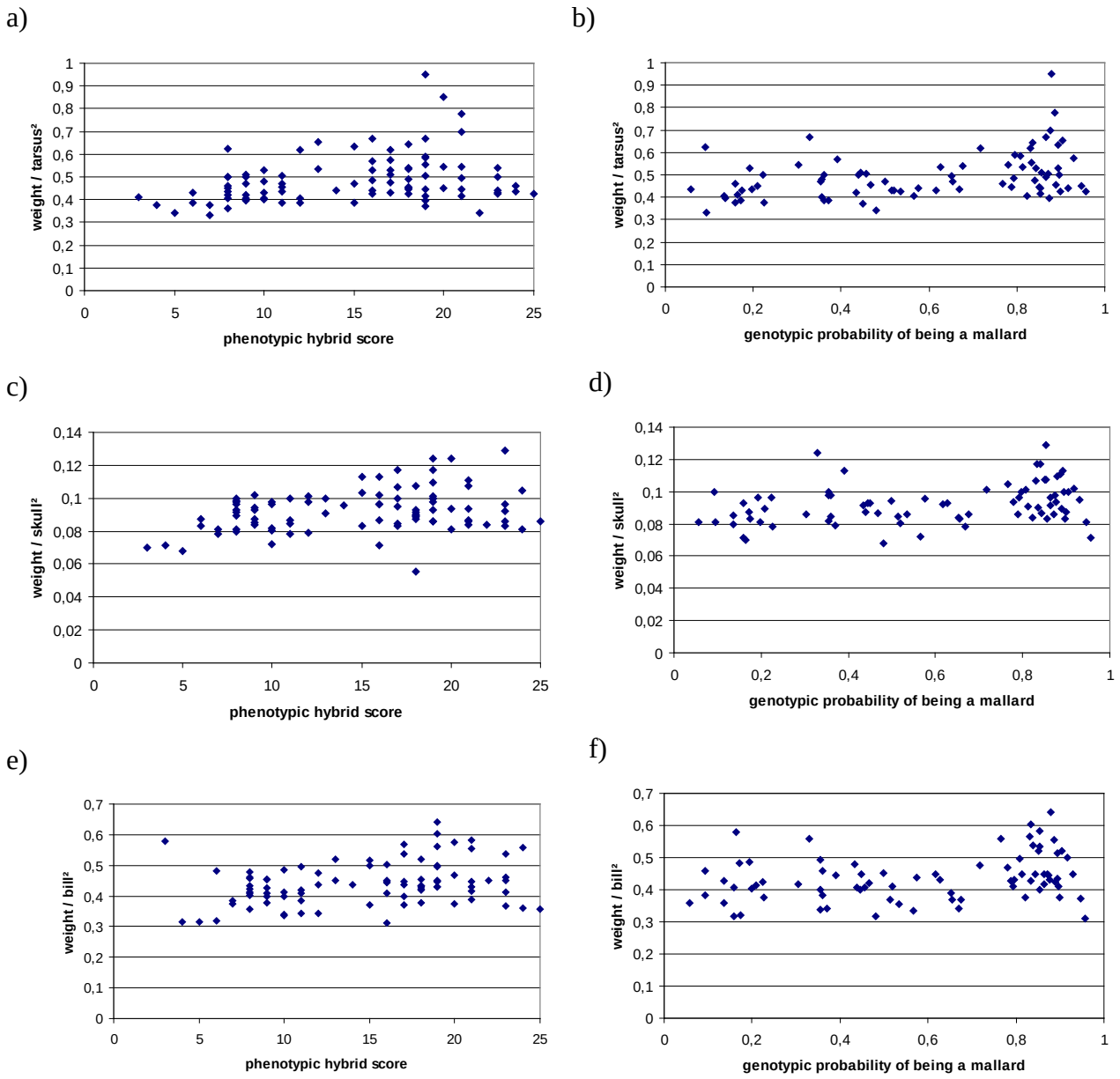
Correlation of measures

The different measures of fitness I used were not independent of each other. Using the subset of 25 live caught ducks from 2007, for which a full dataset is available for all individuals, significant relationships were found between ectoparasite counts and endoparasite presence (ANOVA, p=0.012, F=7.41, df=22), between endoparasite presence and white blood cell counts (ANOVA, p=0.020, F=6.28, df=23), and endoparasite presence and PHA immunochallenge reaction (GLM controlling for swelling of control wing, p=0.014, F=21, df=7.13). I also found significant relationships between ectoparasite count and relative body mass (regression: p=0.004, F=10.29, df=22)

and ectoparasite count and PHA immunochallenge reaction (glm controlling for swelling of control wing: p=0.008, F=8.71, df=20).

Body condition

There was a significant relationship between relative body mass, measured as weight/(tarsus length)^2, weight/(skull length)^2, or weight/(bill length)^2, and the hybrid score of ducks, and this held when I used either phenotypic scores and genetic scores to assess degree of hybridisation (regressions, tarsus vs phenotype: p=0.002, F=10.17, df=76, adjusted r^2=10.6%, tarsus vs genetic scores: p<0.001, F=13.118, df=76, adjusted r^2=14.7%; skull vs phenotype: p<0.001, F=14.41, df=75, adjusted r^2=15.0%, skull vs genetic scores: p=0.003, F=9.291, df=75, adjusted r^2=11.0%; bill vs phenotype: p=0.015, F=6.21, df=76, adjusted r^2=6.3%, bill vs genetic scores: p=0.008, F=7.481, df=76, r^2=9.0%). Wing length measurements were taken but discarded as unreliable due to feather damage. In all significant cases, mallard-like individuals tended to be heavier, but as the low values of r^2 show, only a little of the variation was explained by the genetic or phenotypic score (Figure 1 a-f).

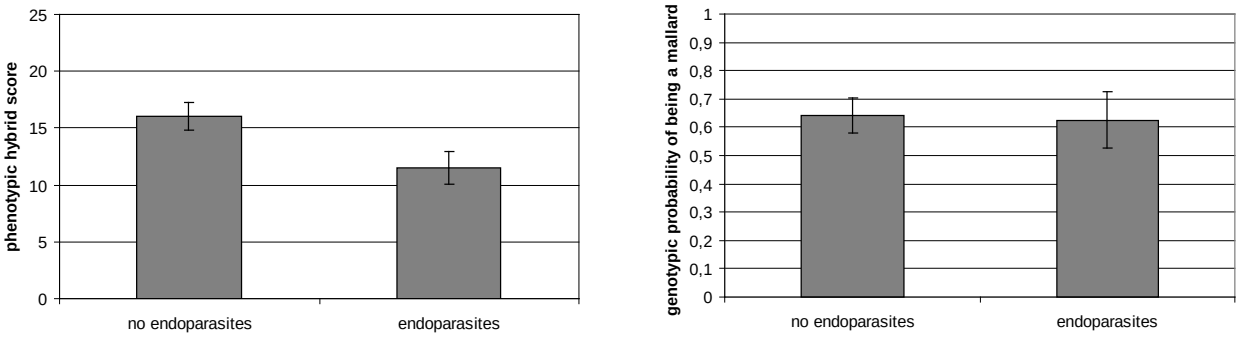


Figures 1 a - f. Relative body mass, calculated as weight per squared length of tarsus, skull and bill, in relation to phenotypic hybrid scores after Rhymer et al. 1994 (left) and genotypic hybrid scores as determined by microsatellite analysis (right).

Parasites

Endoparasite presence was examined for 25 ducks, all in the same season. Eight of these were found to carry internal parasites, including Ascarids (in 4 individuals), Capillaria (3 individuals), Trichuris (2 individuals), Coccidia (1 individual), and Strongyloides (1 individual). Although sample size was small, there was a significant difference in absence or presence of endoparasites by phenotypic scores (t-test, p=0.035, T=2.27, df=19), but none by genetic score (t-test, p= 0.633, T=-0.628, df=23) (Figure 2 a-b). As phenotypic scores are not reliable measures of hybrid status in individuals, the result using genetic scores is more likely to be accurate.

a) b)

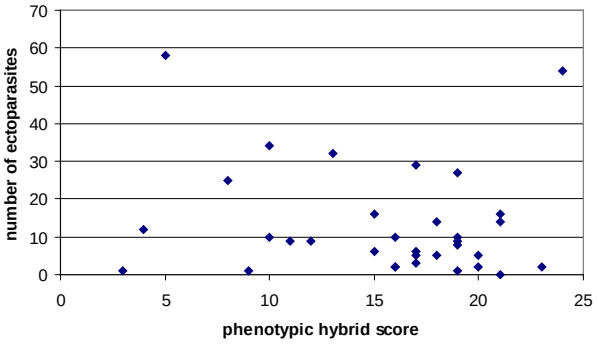


Figures 2 a-b. Average phenotypic hybrid scores after Rhymer et al. 1994 (a) and genotypic hybrid scores as determined by microsatellite analysis (b) in relation to the presence or absence of endoparasites. Error bars represent standard error.

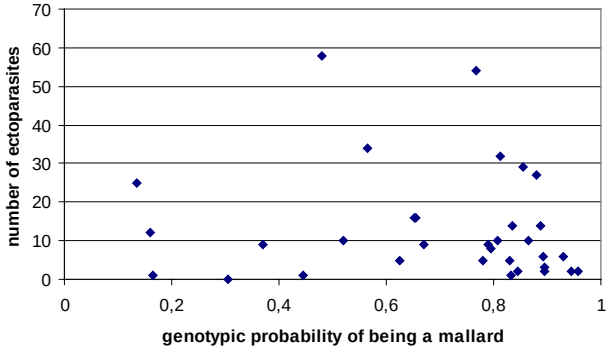
Dust-ruffling of 33 individuals yielded ectoparasites in the four genera of bird lice, Anatoecus and Anaticola, Trinoton and Holomenopon, as well as unidentified mites. Only one bird was free of any ectoparasites, the other samples contained between one and 58 parasite individuals, not counting mites. Few birds carried mites, but one sample

contained 87 individuals. There was no evidence that ectoparasite prevalence varied by phenotypic (regression, p=0.396, F=0.74, df=31) or genetic score (regression, p=0.592, F=0.294, df=31) (Figure 3 a-b).

a)



b)

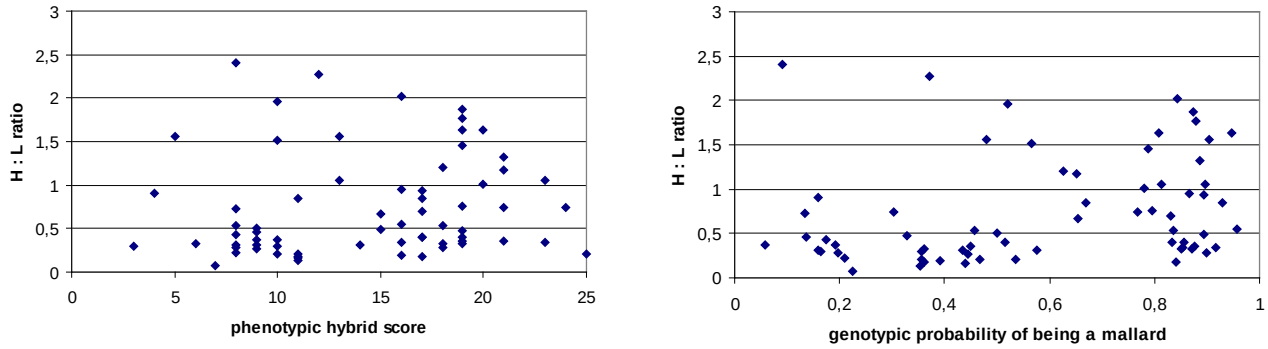


Figures 3 a-b. Number of ectoparasites removed by dust ruffling, in relation to phenotypic hybrid scores after Rhymer et al. 1994 (a) and genotypic hybrid scores as determined by microsatellite analysis (b).

Heterophil : lymphocyte counts

Relative counts of heterophils and lymphocytes did not differ by phenotypic scores (regression, phenotype: p=0.325, F=0.98, df=65), but they did differ by genetic scores genetic score: p=0.049, F=4.014, df=65, r^2=5.8%) (Figure 4 a-b). The analysis using genetc scores is more likely to be accurate than that using phenotypic scores, however, only less than 6% of variation was explained by relative heterophil : lymphocyte count.

a) b)

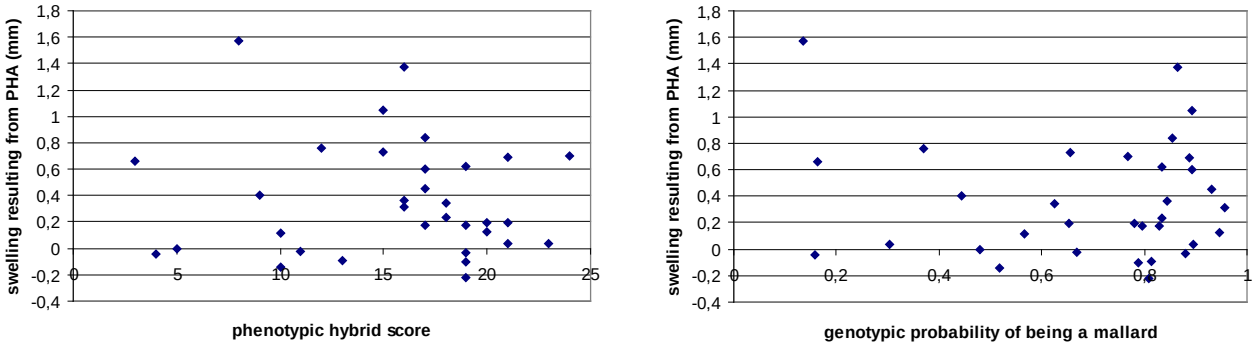


Figures 4 a-b. Heterophil to lymphocyte ratio (H : L ratio), in relation to phenotypic hybrid scores after Rhymer et al. 1994 (a) and genotypic hybrid scores as determined by microsatellite analysis (b).

Immunochallenge with PHA

Skin swelling in reaction to an immunochallenge with PHA did not differ either by phenotypic scores (GLM controlling for swelling of control wing, p=0.575, F=0.32, df=29) or genetic score (GLM controlling for swelling of control wing, p=0.333, F=1.142, df=29) (Figure 5 a-b).

a) b)



Figures 5 a-b. Swelling resulting from PHA injection (calculated as swelling on treatment wing – swelling on control wing for presentation), in relation to phenotypic hybrid scores after Rhymer et al. 1994 (a) and genotypic hybrid scores as determined by microsatellite analysis (b).

Discussion

Summary of results

Condition-related fitness measures were compared between hybrids in a grey duck and mallard hybrid swarm in New Zealand, in which some individuals were more grey duck-like and others more mallard-like. Significant differences between grey-like and mallard-like ducks were found only in body condition, in endoparasite prevalence, when the latter was tested against phenotype, but not genotype, and in heterophil : lymphocyte ratio, when tested against genotype, but not phenotype. The positive result in endoparasite levels, being based on phenotype alone, which is not a good measure of hybrid status, should be treated with caution. No differences were found in ectoparasite levels, or an immunochallenge test. I also found correlations between a wide range of the measures taken, which indicates that the measures likely reflect some underlying

common factor, related to fitness, although the nature of that underlying factor cannot be determined with certainty.

Correlations between the different measures of condition suggest that individuals appearing healthier, as in having less parasites, a more severe PHA response and a lower lymphocyte count, might indeed have a better immune system. Alternatively it might simply be the case that they share some unmeasured factor that influences all of them. For example, the presence of one of the factors may make it more likely for an individual to suffer from others. This could be due to a reduced immune response or to the fact that the same behavioural or genetic trait might influence several of these factors – a very social duck, for example, might be more likely to pick up both ecto- and endoparasites, as well as infections. Nonetheless, the correlations indicate that, at the very least, the factors measured are not independent of each other.

It is not clear from the present study if the significant results I found are likely to have biological significance in terms of differences in selection. A single significant result in the number of tests run cannot be considered conclusive evidence, as level of significance of 95% means that 5% of tests return false positives. In the interpretation of measures related to the immune system, it is particularly likely that small differences observed in one measure only have no biological significance [Adamo 2004]. Unfortunately, the sample size for the test of endoparasites was relatively small at 25 individuals, and a larger sample size might clarify this point, but as only the unreliable test using phenotype was significant, a false positive is likely. Sample size for the heterophil : lymphocyte ratio was 66, and the significant result occurred when compared to genotype, but the result was only just significant.

The heavier body of mallard-like individuals might suggest an advantage to hybrids with increasing mallard genotypes, but this is uncertain as mallards are of a more heavy build than grey ducks. This means that even were the bones scaled down to the size of a grey duck, mallards are heavier. The data might well reflect this difference between the parental species rather than any actual difference in the condition of individuals. Thus, on its own, a linear difference in body size cannot be used to measure the fitness of these hybrids. A non-linear relationship indicating a difference of intermediate hybrids from either pure parental species or hybrids closer either parental species would have been more enlightening. Perhaps a more direct measure of body condition, maybe in destructive sampling, could be used to determine if hybrids hold an advantage once underlying differences in the build of the parental species are controlled.

This study highlights why it is important to use several measures of condition in a study, to prevent mis-interpretation due to a limited range of measurements. In conclusion, there is no convincing evidence that there are any fitness differences in the measures taken between mallards, grey ducks, and their hybrids in New Zealand, although there is some indication that possibly, mallard-like hybrids might carry fewer endoparasites, have a higher heterophil : lymphocyte ratio or be of better body condition than grey duck-like hybrids.

Limitations of the study: restricted sample range

The sample of birds in this study consisted exclusively of hybrids, even if two individuals were of pure mallard phenotype. Despite asking hunters for any potential pure grey ducks, no such ducks could be obtained in two hunting seasons, nor in two seasons

of trapping live ducks. This severely restricted the interpretation of the data collected, but simply reflects the reality of the present hybrid swarm, in that few pure individuals of either parental species still exist. It is probably too late to find any pure New Zealand grey ducks for comparison, as most of the population is now of hybrid origin (see Chapter 2). Nonetheless, I was able to sample a wide range of hybrids that varied from very mallard-like to very grey duck-like, and if any patterns were present within the hybrid swarm, one would expect to see them in this sample.

The uncertain link between immune measures and fitness

My conclusion of no significant differences in condition between grey duck-like hybrids and mallard-like individuals is based on the assumption that the measures used reflect the underlying health of an individual. The link between fitness and immune responses is not simple [Norris and Evans 2000, Adamo 2004], At present, evidence of a link is circumstantial as fitness and immunocompetence have been shown to covary, but this might be due to common, underlying factors [Norris and Evans 2000]. However possible differential investment of the same individuals into different parts of their immune system makes it advisable to always investigate several measures in the same study and to interpret them in context [Norris and Evans 2000, Adamo 2004]. The following paragraphs detail some of the problems with the individual measures used here, and what they mean to the interpretation of the data presented.

Interpretation of data: parasites

The interpretation of the parasite data presented here is based on the assumption that the organisms scored as parasites in this study are indeed harmful to the host. In general, it is accepted that parasites can be harmful, but this does not necessarily mean they have to be. In most cases, when parasites occur in small numbers, infections are asymptomatic [Ebert 1984, Toft 1991]. However, the increased immune response observed in birds infected with parasites such as the protozoan *Eimeria* spp, or Ascarid worms, suggests a cost even if the birds are asymptomatic [Davison et al. 2008], and in the case of injury or similar stresses, the number of parasites can increase dramatically and become a problem [Ebert 1984]. Furthermore, trade-offs between reproductive effort and the costs of the immune system have been shown [Davison et al. 2008]. While this evidence is not conclusive, and every host-parasite relationship is unique, some cost to most parasite infections can be assumed [Davison et al. 2008]. It has been suggested that this cost is large enough to give a marked advantage to species in novel environments, where specific diseases and parasites are absent [Lee and Klasing 2004].

The potential effect of the parasites I recorded has not been studied, although it is known that some of these organisms can be harmful. For example, *Trinoton spp.,* are bird lice that live on feathers and blood, and one is known to transmit heartworm in swans and occurs in higher numbers on individuals weakened by injury or poisoning [Seegar et al. 1976, Cohen et al. 2008]. A bird louse of the genus Holomenopon can cause a lack of waterproofing of feathers in ducks, resulting in chilling and pneumonia in severe cases [Humphreys 1975]. Similarly, haematophagous arthropods can limit the growth and survival of fledgling cliff swallows [Chapman and George 1991], or elicit behavioural

responses by adults to avoid excessive exposure of their offspring [Loye and Carroll 1991]. In grouse, a nematode appears to limit populations in cycles [Hudson and Dobson 1991].

Given the presence of harmful parasites, it also is not clear if higher numbers of parasites indicate a weakened, less healthy individual. Individuals without endoparasites do not necessarily have a better immune response, but might not have been exposed. The low rates of infection I found on ducks necessitated a simple analysis that considered only the absence or presence of any endoparasites, without regard for what type of endoparasite it was or to whether it was present at high or low levels. It is questionable though if all endoparasites are equally virulent. However, for ectoparasites almost all ducks did carry individuals of several species, so exposure can be assumed to have been nearly universal.

Some birds might have lower resistance against parasites, but a higher tolerance against their effects. Thus, despite having more parasites on them, they might suffer less ill effects [Raberg et al. 2009]. While rarely studied, such a relationship has recently been shown in mice infected with malaria [Raberg et al. 2007, Boots 2008]. Again, nothing is known about the species in my study. If there are differences in tolerance, this might mean that some individuals do indeed suffer greater detrimental effects than others despite a lack of difference in parasite numbers. Only further detailed studies could show such a relationship, which however is not indicated by any of the results shown here.

Interpretation of data: phytohaemagglutinin

Injecting phytohaemagglutinin, or PHA, a foreign protein which elicits a T-cell mediated immunity response, is a well established test of a non-specific immune response in avian studies [Smits et al. 1999]. It has been shown that a strong response predicts successful establishment of new populations [Møller and Cassey 2004], and meta-analysis has indicated that PHA, as well as a range of other immune response tests, is a good predictor of survival [Møller and Saino 2004]. This measure is therefore considered to be a relatively likely good indicator of underlying health. However, like all immune measures it is best used in combination with other indicators. In fact, it has been shown in one case that response to PHA was reduced while other components of the immune system were increased in environments that contained a large number of parasites [Lindström et al. 2004]. This appeared to be due to trade-off between different parts of the immune system measurable at the population level [Lindström et al. 2004]. This stresses, once more, why it is necessary to study a range of health measures instead of just one, although nothing in the data presented here indicates such a possible trade-off.

Interpretation of data: Heterophil : lympthocyte ratio

Relative heterophil : lympthocyte counts can be a difficult measure to interpret, as they react to the infection status of an individual at the time sampled. Low lymphocyte numbers may reflect poor immunocompetence, but alternatively they might show a lack of current infections [Norris and Evans 2000, Davison et al. 2008]. The two possible interpretations are impossible to tell apart. Therefore, the difference between the two groups in heterophil : lymphocyte ratios could mean that grey ducks, which have more

lymphocytes, have poorer immunocompetence, or it could mean that they have a lower rate of infections.

Interpretation of data: body condition measures

Assessing body condition non-destructively generally yields less accurate results than destructive sampling in birds as well as mammals [Johnson et al. 1985, Winstanley et al. 1998]. The rest of this discussion will focus specifically on birds and studies done in them. Within non-destructive sampling, there has been considerable debate over what measures and statistical procedures to use, and this debate is far from resolved [Ardia 2005, Green 2001]. More complex measures, such as residual regressions and models using multiple measures have been suggested over simple models like ratios, and it is indisputable that simple ratios can suffer problems [Ranta et al. 1994, Jakob et al. 1996, Blem 1984]. However, from the direct comparison of measures there is evidence that complex indices are not necessarily more accurate than simple ones in predicting body lipid content, and, surprisingly in particular body mass, the simplest of all measures, can be the most accurate measure [Spengler et al. 1995, Ardia 2005, Schamber et al. 2009].

Ideally, a pilot study should be conducted for each species to determine the best measure [Spengler et al. 1995, Schamber et al. 2009]. However, this is unrealistic in most scenarios. Here, ratios rather than body mass were chosen due to the very large (factor 2) size range within the sample [Johnson et al. 1985]. To make the conclusions more robust, several measures were taken. As discussed above, the differences I found between more grey-like and more mallard-like ducks probably reflect underlying species differences in build, rather than fitness differences.

Implications for the hybrid population

While each of the measures has its limitations, a cautious interpretation for the combined results is that there is no measurable condition difference within the hybrid swarm, between individuals similar to one parental type or the other. There is some indication that grey duck-like individuals might have higher endoparasite prevalence, and lower numbers of lymphocytes, which might be indicative of a poorer immune response. Therefore, if there is a difference in condition within the hybrid swarm, grey-like individuals appear to be doing worse than mallard-like individuals.

It is possible that a lack of health-related variation connected to phenotypes or genotypes is due to the fact that most, if not all, individuals caught are likely to be later-generation hybrids. It is known that genes conferring adaptive advantages can selectively introgress in hybrid zones [Martinsen et al. 2001, Stein and Uy 2006], and it is reasonable to expect that with the very large amount of genetic variation in hybridising populations, and the resulting high variance in fitness levels, selection may act very swiftly at least on simple genetic traits. Therefore, the swarm might already have experienced significant selection towards the fittest genotypes. Possibly, grey ducks and earlier generation hybrids did suffer worse condition. Alternatively, it is possible that these two species never differed much in their condition in New Zealand. Hybrids of the closely related American black duck (*Anas rubripes*) and the mallard (*A. platyrhynchos*), in comparison, appear to suffer an increased parasite load compared to either parental species [Mason and Clark 1991]. In the end, given the extensive nature of hybridisation, it is probably not possible any longer to investigate if the grey duck and mallard ever did differ in health-related fitness in New Zealand, but further studies might clarify if a difference exists

between more grey duck-like individuals and more mallard-like individuals within the current population.

In any case, the lack of a pronounced difference would favour further mixing as hybrids do not appear to suffer negative effects in this measures that might have slowed hybridisation. If the positive results presented here should be real, they appear to favour the mallard-like genotypes, and thus would put the grey-like types at a selective disadvantage.

Non health-related measures

Measures beyond the scope of this study might very well differ between more mallard-like and more grey-like ducks. It is known that reproductive output varies markedly between these species, with mallards being significantly more fecund, and hybrids being intermediate to the parental species [Williams and Roderick 1973]. Differences in mortality, especially in mortality mediated by hunting, have also been studied. While some studies indicate that mallards might have lower mortality than grey ducks, other studies found no such effect [Caithness et al. 1991, Williams and Basse 2006]. Mortality might vary considerably regionally and temporally, making it hard to arrive at a reliable national estimate.

Inclusion of several more measures had initially been planned. Amongst this was the measurement of semen quality, which was not possible as no permit was granted to capture or collect male ducks during breeding season. Measurements taken in the first field season to investigate fluctuating asymmetry also failed to meet reliability criteria in the traits selected [van Dongen et al. 1999, Lens et al. 2002].

Conclusion

A lack in health differences between mallards, grey ducks and their hybrids in New Zealand could help explain the widespread presence of hybrids within the country, and does suggest that hybrids at least are not at a pronounced disadvantage. If condition related factors do play a role, they are likely to favour mallard-like hybrids over grey-like ones. The results presented here further confirm the prognosis that hybrids between these species will continue to spread, unhampered by major selective disadvantages.

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Chapter 6

A mathematical model of hybridisation: can the fates of populations be predicted?

Abstract

A stochastic, individual-based model was constructed to simulate hybridisation between two populations newly brought into contact. It was tested with data on two examples of hybridising populations that differ in their degree of introgression: (1) limited hybridisation between native red deer (*Cervus elaphus*) and introduced sika deer (*C. nippon*) in the Scottish Highlands, and (2) widespread hybridisation between native grey duck (*Anas superciliosa*) and introduced mallard (*A. platyrhynchos*) in New Zealand. The model successfully predicted both limited hybridisation in the deer and widespread hybridisation in the ducks. Sensitivity analysis of the grey duck and mallard hybridisation suggests that differential reproductive output and the degree of assortative mating are the most important factors influencing the degree of hybridisation and the proportionate amount of genetic material of each species in the total population. In contrast, the effect of survival on degree of hybridisation was negligible. Within the ranges of likely variables, almost every scenario led to widespread hybridisation between the two duck species. This suggests that it is unlikely for any populations of grey duck and mallard to coexist over the long term without significant hybridisation. My model supports field observations that populations of the grey duck might be at risk from hybridisation everywhere that mallards have been introduced into their range, including Australia, and thus possibly threatening the genetic integrity of the species across most of its range.

Introduction

Hybridisation is a conservation problem of increasing importance worldwide [Rhymer and Simberloff 1996, Woodruff 2001, Seehausen et al. 2008]. While hybridisation can be a natural evolutionary process [Arnold 1997, Dowling and Secor 1997, Arnold et al. 2001, Levin 2002], it can also be induced or increased by human activities when species are introduced into the ranges of species that were previously allopatric, if species extend their ranges due to human activities, if alteration of habitat results in increased contact between sympatric species, or if one species is reduced in numbers to the degree that they might not encounter mates of their own species [Rhymer and Simberloff 1996, Mooney and Cleland 2001, Levin 2002, Seehausen 2006, Seehausen et al. 2008]. Such hybridisation can threaten the integrity of species and lead to extinction [Rhymer and Simberloff 1996, Woodruff 2001, Levin 2002].

The conservation consequences of hybridisation are difficult to predict. Outcomes can range widely, from the occurrence of occasional sterile or inviable offspring with no further consequences at one extreme, to large scale introgression and the formation of hybrid swarms and extinction of one or both participating species at the other extreme [Arnold 1997, Barton 2001, Burke and Arnold 2001, Levin 2002, Seehausen et al. 2008].

Effective conservation management of hybridisation usually involves the prevention of further hybridisation and this is often most successful and most cost effective when hybrids first occur, and are found at low frequencies. For example, hybridisation between the critically endangered black robin (*Petroica traverse*) and the sympatric Chatham island tomtit (*P. macrocephala*) was eliminated by temporarily removing the latter species and creating allopatric populations [Butler and Merton 1992]. In those cases

where a hybrid swarm has formed, species can no longer be conserved other than as an admixed unit. This has happened, for example, between native and introduced tiger salamanders in California [*Ambystoma californiense and A. tigrinum*; Riley et al. 2003] and between two pupfish species and the introduced sheepshead minnow [*Cyprinodon pecosensis*, *C. bovines*, *C. variegates*; Wilde and Echelle 1992, Childs et al. 1996, Echelle and Echelle 1997].

Given variation in the degree to which hybridisation has been observed, it is not surprising that it has been difficult to predict whether hybridisation in any given species pair and/or set of circumstances will develop into a problem and how large a threat it may become. As a consequence, effort and resources may be wasted on the control of hybrids that would never have developed into a conservation threat, while in other cases hybrid control may start too late to prevent irreversible consequences [Allendorf et al. 2001]. Increased understanding of the processes determining the outcomes of hybridisation is therefore highly desirable for the wise use of conservation resources.

Mathematical models can be one tool to help increase our understanding of complex biological processes, and to generate testable predictions of the potential outcomes of such processes. Models can be employed to address a variety of aims in the context of hybridisation – for example to understand the evolutionary significance of hybridisation, to better understand a specific case of hybridisation, or to understand the ecological or geographic dimension of hybridisation. Different models are generally required for each aim, and depending on the precise purpose, they may contain variables describing geographic circumstances, life history characteristics, genetic or evolutionary processes,

and so on. Models can also be either deterministic or stochastic, depending upon whether general patterns are sought or variability and uncertainty are to be explored.

Several modelling approaches have been employed with regard to hybridisation in a conservation context. For example, Seehausen et al. [2008] used models to predict the consequences of increased hybridisation due to human activities at the scale of overall biodiversity, and found that as long as extinction rates remain high, hybridisation would lead to a further decrease of diversity. Several models have been written to predict the risk of extinction for a plant species by hybridisation with an introduced species, and the amount of introgression likely to occur under several sets of assumptions [Huxel 1999, Wolf et al. 2001, Hall et al. 2006]. A model was also used to predict the risk to newly established red wolf (*Canis (lupus) rufus*) populations by hybridisation with coyote (*C. latrans*), and evaluate a range of management options [Fredrickson and Hedrick 2006]. However, to the best of my knowledge no model has been published that was (1) generally applicable to many animal cases, rather than one specific case, (2) allowed for the inclusion of variables describing mortality, reproduction, and survival, as well as be (3) stochastic and (4) allowing for sensitivity analyses. In particular, there is a need in the conservation literature to develop such a stochastic model that also provides the possibility of running sensitivity analyses to analyse uncertainty and to uncover possible gaps in the knowledge about a case.

Native grey ducks (*Anas superciliosa*) and introduced mallards (*A. platyrhynchos*) are two species that hybridise widely in New Zealand (see Chapter 2). The extent of the hybridisation threatens the integrity of the native species, and a hybrid swarm appears to have become established nationwide [Williams 1998, Williams and Basse 2006] with

very few remaining pure grey ducks. The mallard has also been introduced into most other parts of the range of the grey duck, most importantly to Australia (the largest single population), and to most Pacific islands [Marchant and Higgins 1990]. Therefore, hybridisation may threaten the grey duck across its entire range. Previously, the risk of widespread hybridisation was judged low in Australia, as the dry interior was thought to be less favourable to mallards [Braithwaite and Miller 1975]. However, hybrids were already being encountered in Australia's cities at the time of that study, and mallards are now widespread in the southeast and along coastal Australia [Marchant and Higgins 1990].

Given the risk of hybridisation to the survival of the grey duck, modelling might prove to be a useful tool to help establish details of the size and nature of the risk of widespread hybridisation in areas of sympatry with the mallard. Furthermore, modelling can be useful for determining the influence of factors such as lower survival or reproductive rates (or other life history traits) on hybridisation and how this may limit the spread of a hybridising invasive species. In this chapter, I used modelling to address hybridisation. First, I present a mathematical model that simulates the formation of a hybrid swarm, taking into account the relative fitness of parental species and hybrid classes, differential reproductive potential, survival, and assortativeness of mating. Next, I show that this model adequately predicts different outcomes when run with the data for two different hybridising species pairs, which show different patterns of hybridisation in nature. Lastly, with the use of sensitivity analysis, I show which parameters were most relevant to explaining the observed patterns of hybridisation between grey duck and mallard, and thus, the circumstances under which hybridisation is to be expected.

Methods

Description of the mathematical model

A stochastic, individual-based model was written in Matlab (7.1). It simulates a population of individuals at carrying capacity over a given number of discrete generations (G), starting with two discrete species which may admix during the simulation. The complete code can be found in the appendix.

The initial population was determined by the total population size (S), and the proportion of this total population that belong to species 1 (SP). The remainder was automatically assigned to species 2. In this model, species 1 can be viewed as the native species and species 2 as the invasive or introduced species, although the model applies just as well to two native hybridising species. Each individual of species 1 was assigned a genetic value of 0, signifying that it carries 0% of the genome of species 2, while all individuals of species 2 were assigned a genetic value of 100%. Two groups of data were generated, representing males and females, which were assumed to make up half of the population (i.e. a 50/50 sex ratio).

Males and females were initially “paired” randomly with regard to their genetic value. If pairs had the same genetic values they accepted each other and attempted to breed. Pairs with dissimilar values also accepted each other and bred, but only if the dissimilarity of their genetic values was less than a threshold level (GD). If two individuals were more dissimilar than the threshold, they paired only with a specified likelihood (LH). Any rejected individuals paired again randomly. This process was repeated three times, and any individuals not paired at the end of this period remained

unpaired. This approach allows researchers to define the level of assortativenss in mating, both in regard to which individuals are accepted as conspecifics, and the probability with which heterospecific partners are accepted.

The reproductive output of breeding pairs was determined according to the genetic value of the ‘female’. The average reproductive output of species 1 (R1) and species 2 (R2) were set, and a common variance (V) was also set. The average reproductive output of females with intermediate genetic values was proportionate to their genetic value. Previous studies using captive breeding have shown that hybrid females between grey duck and mallard lay more eggs if they have a higher proportion of mallard-derived genome [Williams and Roderick 1973]. A modification might be necessary to apply the model to other cases. On the other hand, there is no known difference in reproductive output between red deer, sika deer, and their hybrids, so reproductive output in this case is not affected by proportionate genetic value.

To determine offspring number for each pair, a value was drawn from a distribution with the mean determined by the female’s genetic value (i.e., R1 for a species 1 female, R2 for a species 2 female, and intermediate for a hybrid female) and the variance as specified (V). The genetic value of each offspring was intermediate between the parents.

Mortality of young was dependent on their genetic value. This section is flexible and can be changed for each hybridisation case modelled. For all runs presented here, any individual within a specified value (TSS) of either ‘pure’ form (i.e., with genetic values of 0+TSS%, or 100-TSS%) was accepted as ‘pure’, and suffered a mortality for species 1 (T1) and for species 2 (T2). All remaining individuals suffered the mortality of hybrids (T3). In this way, hybrids can be set to suffer more, less, or intermediate levels of

mortality compared to parental types, and the two parental types can also differ. Additionally, the threshold value (TSS) can be used to determine that hybrids with limited introgression do not suffer mortality different from the parental type they resemble. Surviving individuals were then randomly assigned to a sex, reduced to the specified population size, and this was the starting population for the next generation.

Output from the model was provided in the form of genetic composition of the overall population at the end of each generation. The program can provide histograms of these data, and save averages and standard deviation of the histograms of selected generations of each trial to a separate file. Due to the large amount of data contained in a series of histograms, the latter is usually the most practical form for data storage and analysis.

Test of the model on ducks and deer

While the model was developed with the grey duck and mallard in mind, it should also be applicable to other cases of hybridisation, and I tested this using data on hybridisation between red deer (*Cervus elaphus*) and sika deer (*C. nippon*) in Scotland [Abernethy 1994, Goodman et al. 2008]. First imported around 1880, sika deer later became established in the wild where the population grew, and from 1950 onwards reports of hybrids were common. In Argyll, Scotland, sika deer have been present now for about 20 generations, the population is expanding, and hybrids are reported where the two species co-occur. Nonetheless, the two populations remain clearly distinguishable despite the occurrence of introgression [Abernethy 1994, Goodman et al. 2008].

In contrast to the limited hybridisation evident between the two species of deer in Scotland, the species barriers appear to have broken down between grey duck and

mallard in New Zealand, and a hybrid swarm has become established [Williams 1998, Williams and Basse 2006, also see Chapter 2]. Using the two extreme cases, I gathered data available from the literature to test if my model could accurately predict the different outcomes between the two cases. The data used are presented in tables 1 and 2. For information on hybridisation in deer I used the following sources: Clutton-Brock et al. [1982], Abernethy [1994], Long [2003], Goodman et al. [2008]. For information on hybridisation in ducks I used: Williams and Roderick [1973], Marchant and Higgins [1990], Caithness et al. [1991], Williams [1998], Heather et al. [2000], Drilling et al. [2002], Williams and Basse [2006]. The results of Chapter 3 of this thesis were also used to assess the two parameters relevant to mating patterns, namely the genetic distance to which an individual is accepted as a conspecific, and the likelihood of a conspecific mating.

Table 1. Data used for runs of the model for hybridisation of red deer and sika deer in Scotland, and of grey duck and mallard in New Zealand. For list of data sources consult text.

Parameter	**Abbreviation in model**	**Red and sika deer**	**Grey duck and mallard**
Generations	G	20	50
Population size	S	4000	10 000
Proportion of native species in total start population	SP	0.4	0.94
Genetic distance to which accepted as conspecific in mating	GD	30%	30%
Likelihood of heterospecific mating	LH	0.0015	0.25
Mean reproductive output native species	R1	7.4	8.5
Mean reproductive output foreign species	R2	7.4	11.5
Variance of reproductive output	V	2.5	2
Introgression level tolerated as pure for mortality	TSS	20%	30%
Mortality of native species	T1	21%	57%
Mortality of hybrids	T3	21%	57%
Mortality of foreign species	T2	21%	57%
Number of iterations of model	i	500	500

Presentation of results

Because of the large amount of data generated in each of these model runs, and the limits of the computer system available, only limited data could be stored. For each of the 500 repeat runs, the mean and standard deviation of the histogram of every fifth generation was calculated and stored, and results of the final generation shown.

To present the data, the expected standard deviation for a non-hybridising population with the given mean was calculated using the formula:

$$((\text{mean}*((100-\text{mean})^2))+((100-\text{mean})*((0-\text{mean})^2)))/100)^{0.5}$$

This expected standard deviation was divided by the observed standard deviation. A value of 1 signifies a population with no admixture, while lower values show populations with increasing hybridisation. This measure is referred to as the level of species integrity, and was plotted onto the mean, or proportion of genome in the population belonging to the native species (species 1). This method allows for easier presentation and interpretation, as it fits onto a rectangular grid instead of a half-circle grid.

Sensitivity analysis

By employing sensitivity analysis, the sensitivity of the model to changes in individual parameters can be estimated [Fry and Patil 2002]. A sensitivity analysis can be used to measure which input variables into a multi-factor model most affect the outcome and in what way. To this end, put simply, the model is run with many different input values, and a statistical relationship between input and output is then assessed. This method was used to determine if the available data for the grey duck and mallard were accurate enough to reliably predict a population's hybridisation behaviour. Data ranges of the parameters were estimated using the same sources as above. Care was taken to choose wide ranges of data, including all estimates found in the literature, and to add a margin where estimates were likely to be less accurate.

Table 2. Data ranges for the sensitivity analyses for hybridisation of grey duck and mallard in New Zealand. For list of data sources consult text.

Parameters	**Grey duck and mallard**
Generations	50
Population size	10 000
Proportion of native species in total start population	0.92 – 0.98
Genetic distance to which accepted as conspecific in mating	5 – 30 %
Likelihood of heterospecific mating	0.05 – 0.5
Mean reproductive output native species	8 – 10
Mean reproductive output foreign species	9 – 13
Variance of reproductive output	2
Introgression level tolerated as pure for mortality	5 – 40 %
Mortality of native species	45 – 70 %
Mortality of hybrids	45 – 70 %
Mortality of foreign species	45 – 70 %
Number of iterations of model	500

Five hundred sample sets were drawn from these ranges using Latin Hypercube sampling, following the recommendations and methodology of Saltelli et al. [2000]. In brief, the range of interest was divided into intervals of equal probability, and a random sample was drawn from each range. Samples drawn for each parameter were paired randomly with those of other parameters. Latin hypercube sampling enables multi-parameter analyses of stochastic values with a reasonable number of iterations. Had conventional random sampling been used, a much larger number of iterations would have been required than the 500 used here. As a run of 500 iterations took about 14 days, it was desirable to restrict the number of iterations. Repeated runs confirmed that Latin

Hypercube sampling yielded overall results that were repeatable. These data were then fed into the model. As limited data could be stored, only the means and standard deviation of every fifth generation were calculated and used for presentation.

The results were analysed by general linear model in Minitab (15.1.0.0), to determine what influence the input data for each parameter had on the result [Fry and Patil 2002].

Results

Tests on ducks and deer

Figure 1 shows the results of the model run on the data set for hybridising red and sika deer in Scotland. The model consistently predicted a population with little hybridisation. One of the 500 runs resulted in a pure red deer population after 20 generations as introduced sika deer went extinct, but they persisted in the remaining 499 runs. Most of these (421) showed no signs of admixture, and in the remaining 79 runs hybridisation was limited (Figure 1).

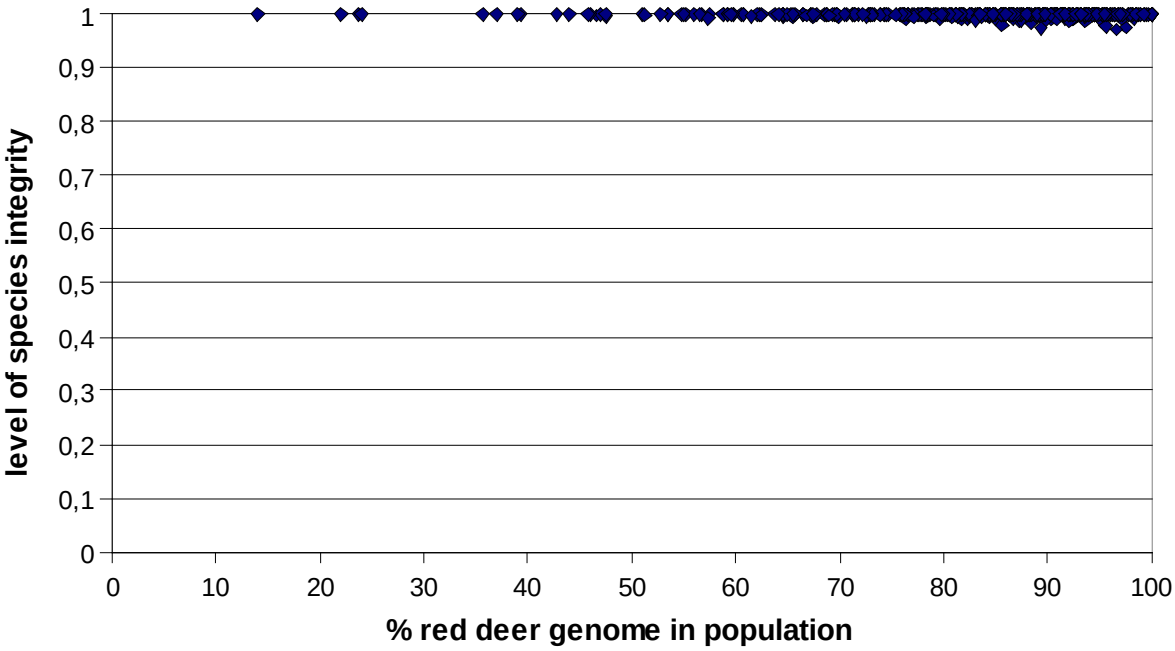

Figure 1. Results from 500 repeated simulations of 20 generations of hybridisation between populations of red deer (*Cervus elaphus*) and sika deer (*C. nippon*). In this simulation identical values for all parameters defining the likelihood of heterospecific mating, reproductive output, and mortality were used (for values see Table 1). A high 'level of species integrity' means little admixture between the two species (for exact definition see text).

In contrast to the low levels of admixture between the two deer, the same model using the grey duck and mallard data set resulted in all 500 runs leading to strongly hybridised populations (Figure 2), all of which were dominated by mallard genes, even if no pure mallards survived. Grey ducks contributed only between 5% and 16% to the total genome of the final hybrid swarm populations.

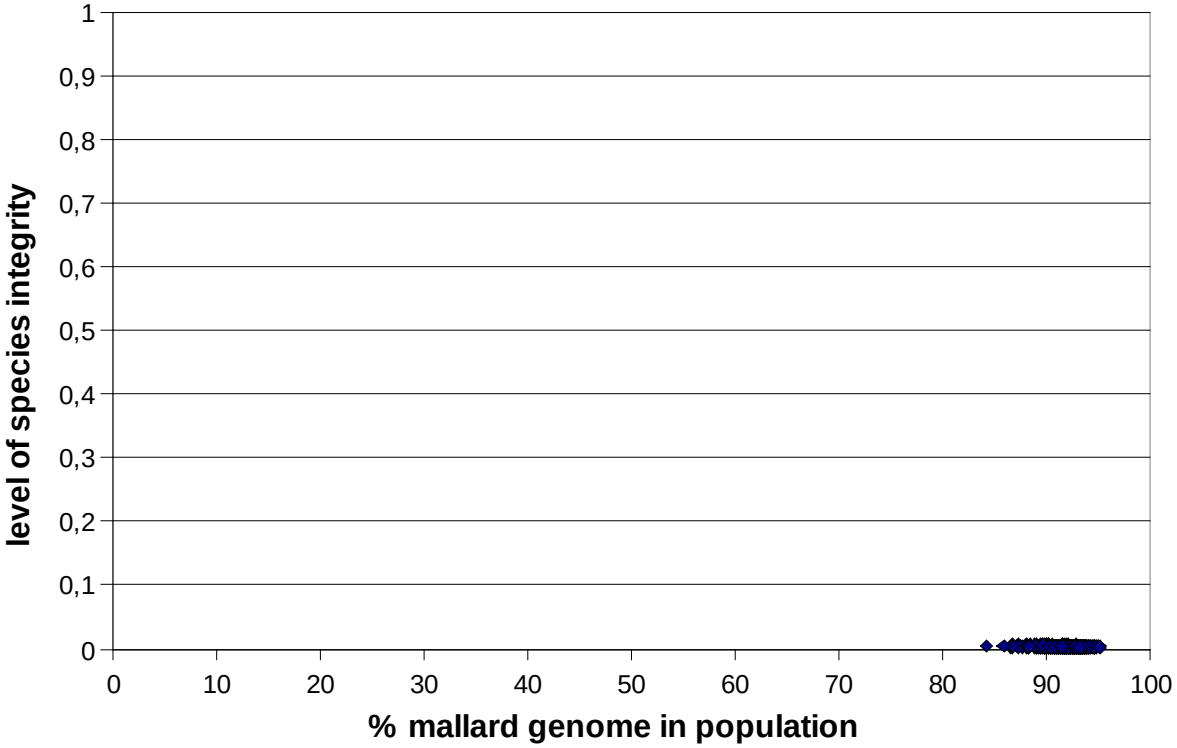

Figure 2. Results from 500 repeated simulations of 50 generations of hybridisation between populations of grey duck (*Anas superciliosa*) and mallard (*A. platyrhynchos*). In this simulation identical values for all parameters defining the likelihood of heterospecific mating, reproductive output, and mortality were used (for values see Table 1). A high 'level of species integrity' means little admixture between the two species (for exact definition see text).

The temporal pattern of hybridisation between mallards and grey ducks is evident by examining the results of the model simulation in stages every five generations during the run (Figure 3). The hybridisation does not proceed linearly, but in two phases. For the first fifteen generations (the first four data points), there is a strong increase in the proportion of mallard genome in the population, but little hybridisation. From the

twentieth generation on, homogenisation of the population increases fast, while the proportion of mallard and grey genome hardly change after the twenty-fifth generation.

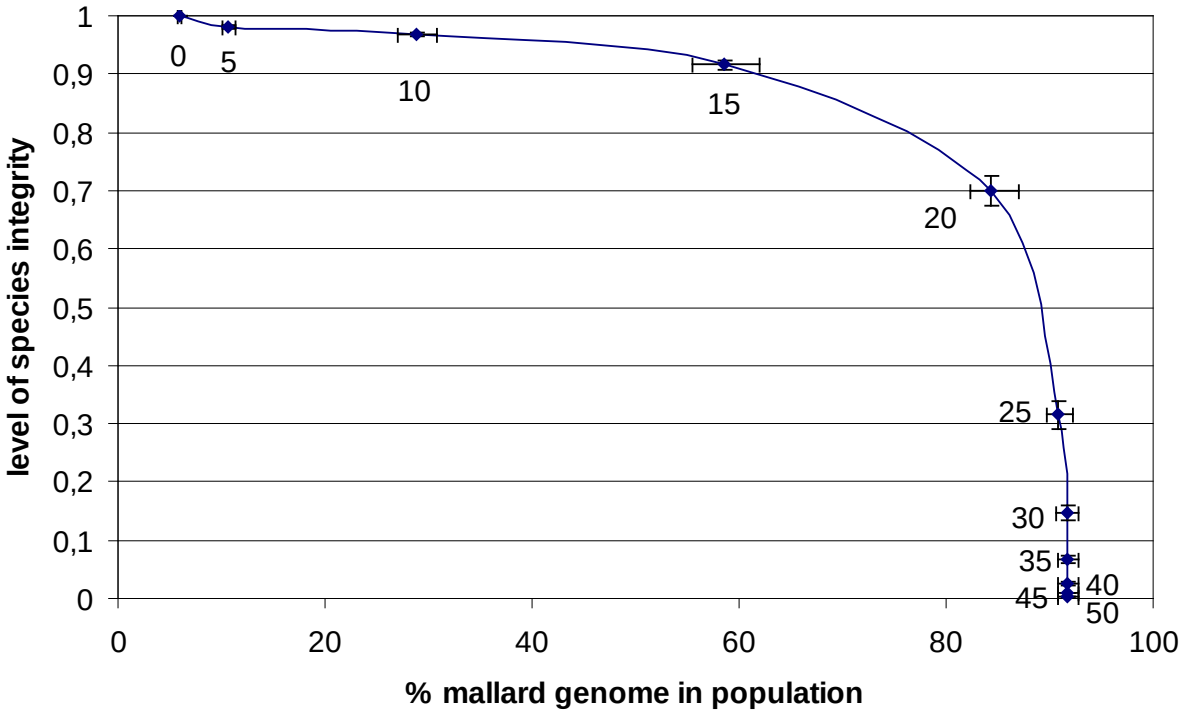

Figure 3. Time series from 500 repeated simulations of 50 generations of hybridisation between populations of grey duck (*Anas superciliosa*) and mallard (*A. platyrhynchos*), using identical values for all parameters defining likelihood of heterospecific mating, reproductive output, and mortality (for value ranges see Table 1). Data points show intermediate results every five of 50 generations (medians and inter-quartile ranges of the 500 runs shown), each marked with the generation they represent and starting with the first generation at the top, marked 0, and ending with generation 50 at the bottom right. For definition of 'level of species integrity' see text.

Sensitivity analysis of the grey duck and mallard hybridisation

Figure 4 shows the results of the sensitivity analysis. In 5 of 500 different scenarios, mallards did not become established. In every other case (99% of runs), admixture occurred, and hybridisation was usually very high and in many cases complete, especially if mallard genome made up more than half of the total population genetic material.

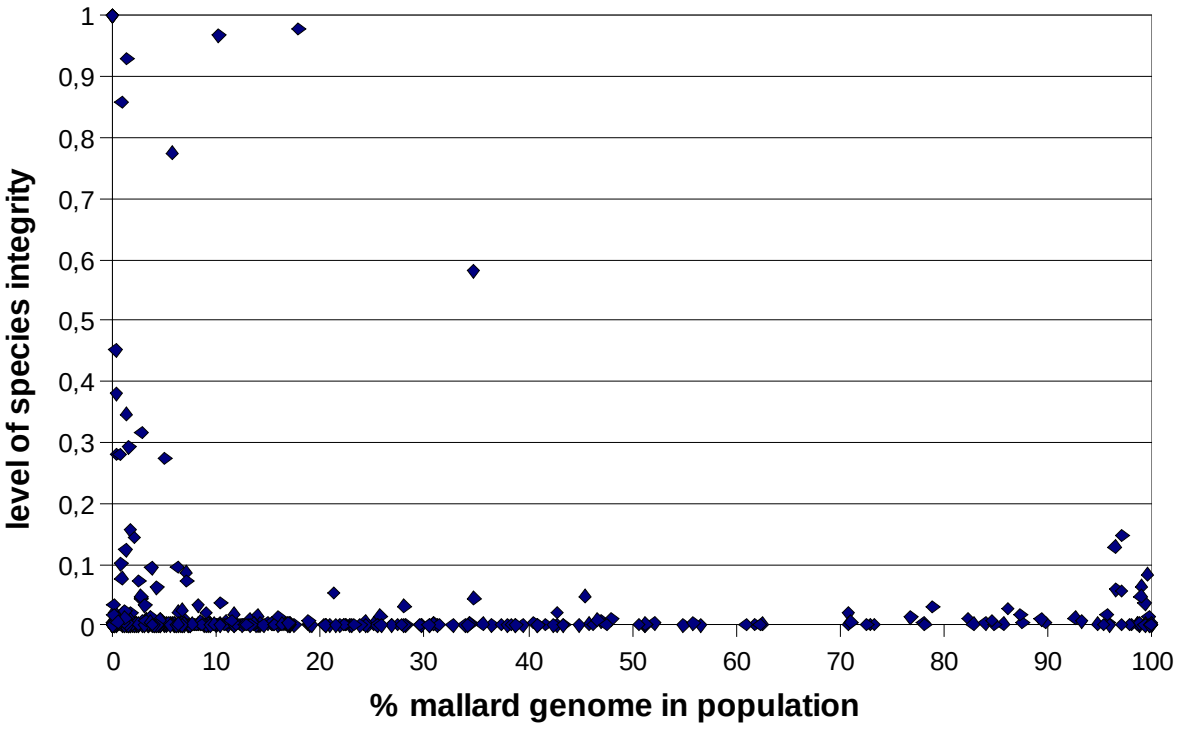

Figure 4. Results of a sensitivity analysis. Simulation of 500 populations of grey duck (*Anas superciliosa)* and mallard (*A. platyrhynchos)* after 50 generations, using different parameter values that define likelihood of heterospecific matings, reproductive output and mortality (for value ranges see Table 2). For definition of 'level of species integrity' see text.

The general linear model showed a significant influence on the average genetic material of the population from (1) the initial proportion of the foreign species, (2) from differences in reproductive output between the species, and (3) in factors influencing assortative mating (all $p < 0.001$). Together, these factors explained two thirds of the variation in results (r^2 adjusted = 68.0). In contrast, differences in mortality played no significant role.

The degree of hybridisation was influenced significantly by reproductive output of both species, and by factors indicating assortative mating (reproductive output of species 1: $p = 0.802$; of species 2: $p = 0.003$; of both in interaction: $p < 0.001$; genetic distance to accept a partner: $p < 0.001$; likelihood of accepting another species partner: $p< 0.001$). These factors together explained one third of the variation in results (r^2 adjusted = 33.3).

Discussion

Summary of results

The model introduced here accurately simulated differences in hybridisation likelihood in two test cases, correctly predicting low levels of hybridisation for red deer and sika deer in the Scottish Highlands [Abernethy 1994, Goodman et al. 2008], but high levels in mallards and grey ducks in New Zealand [Williams 1998, Williams and Basse 2006]. In the duck example, my model predicted a non-linear progression of hybridisation, with limited hybridisation initially but a strong increase in mallard numbers over the first twenty generations, and then a subsequent phase in which the population homogenises rapidly but genome proportions remain almost unchanged. Sensitivity analysis of the grey duck and mallard hybridisation suggests that hybridisation

almost invariably results when mallard and grey duck are brought into contact, but that differential reproductive success and the degree of assortative mating are determinants of the extent of hybridisation, while mortality differences appear to play no significant role.

Causes of differences between the deer and duck cases

The difference between the deer in Scotland and ducks in New Zealand, both in nature and in the model, is probably due to the fact that hybrid ducks eventually become numerous enough to find other hybrid ducks to mate with, and do so with high probability (also see Chapter 3), while deer hybrids might never reach this threshold, thus mediating only limited gene flow between the populations. This could moreover explain why the model shows that at first hybridisation between mallard and grey duck is limited, but increases dramatically as time progresses and a certain number of hybrids is present (Figure 3). While no reliable datasets are available to confirm that this pattern also happens in the wild (in most existing historic datasets, such as the data from Fish and Game on New Zealand ducks, hybrids were not a recognised category), this dynamic has frequently been noted and commented on by hunters (personal communications). This could suggest a threshold effect, where hybrids spread rapidly once their numbers are large enough to effectively form their own population, which nonetheless continues to cross back into parental stock, thus reducing them in number.

Limitations and strengths of the model

As a model, this simulation necessarily simplifies the natural scenario. For example, it does not take into account the degree of habitat homogeneity, only approximates actual

mate choice, and might miss other factors altogether. Ecological factors, such as habitat preferences, while an interesting addition, would have complicated the model to the point of making it infeasible to run on the computers available. It would have necessitated not just an estimate of habitat preference for each species and their hybrids, but also demanded the inclusion of an estimate of the available habitats and their carrying capacity, and possibly change over time. Ideally, it would be a spatial model, allowing for separate developments in different populations, with a certain amount of migration. This was beyond the scope of this study. Instead, different scenarios with different mortality, reproductive rates and degrees of assortative mating can be seen as representations of different habitats, or habitat combinations. For example, a scenario with a high level of assortative mating may represent one where species prefer different habitats and thus rarely meet.

Furthermore, a model is only ever as good as the input data, which in the two cases studied here may be subject to errors or may not be known with sufficient accuracy. This concern is particularly relevant to the data used for the two initial case studies, where a median had to be chosen from available literature, and estimates had to be taken with limited information. However, these tests were predominantly run as validations of the model, to assess if the model could predict the differing results in these two cases of hybridisation, while the sensitivity analysis was run to obtain insights into the grey duck and mallard hybridisation. Therefore, the data ranges for the sensitivity analysis were chosen to be very broad and include all published estimates or measurements, in cases of poorly understood factors with a wide margin of error. This bears the risk that the sensitivity analysis will overestimate the influence of factors that are poorly known and

thus have a wide range, but limits the risk of missing any scenarios likely to occur in nature.

Despite the potential limitations, a model does allow one to test the completeness and accuracy in the understanding of a process. If all important factors are known and included in the model, then a model should accurately reflect reality. If it does not, some influential factor or factors have been measured without sufficient accuracy or are being missed altogether. Here, it appears that the model performs well in overall outcome, predicting very different results for the two cases. These accurate predictions indicate that our knowledge, while not perfect, is probably sufficient to understand the broad mechanisms underlying the hybridisation processes in these two cases.

The model was written to be flexible enough to accommodate a wide range of hybridisation cases. Modifications are possible to model different patterns of differential mortality or reproductive output, or mating patterns. Thus, my model might be helpful to investigate other cases of hybridisation. The main disadvantage is the long run-time on a computer of one or two weeks for large populations, and the large amount of data generated can be difficult to manage. Due to the stochastic nature of the model, the output is also rarely very precise. This is likely, however, to reflect the stochastic nature of natural hybridisation processes, which are not fully deterministic. In other words, bringing together the same species twice can result in different outcomes, and this model reflects such uncertainty, as the output presents what is hopefully the full range of possible outcomes of an encounter. It is therefore strictly necessary to run the model a large number of times for each scenario tested, and to consider the full range of outcomes. Despite this stochasticity, the results in these examples agree on key points,

such as the lack of widespread hybridisation in the deer, and the predominance of the mallard genome in the total hybrid population of the ducks.

The probability and nature of hybridisation between grey duck and mallard

The main result of the sensitivity analysis was that within all data ranges tested, which were chosen broadly as to ensure inclusion of all likely conditions in nature, hybridisation between mallard and grey duck resulted. This indicates that in any form of contact, under any conditions likely to occur, the two species will hybridise widely sooner or later. Long-term coexistence of the two species is not to be expected, no matter what the circumstances, unless these circumstances are of an usually extreme nature and thus fall outside the ranges considered here. However, the ensuing hybrid swarm might be more or less dominated by mallard genome, depending on some of the parameters. Nonetheless, stochasticity also plays a role, and the influence of parameters, discussed below, should be seen as modifiers, which while having an influence, are probably not large or consistent enough to be recommended as management instruments.

Sensitivity analyses tend to find the largest influence in those factors known with least precision, and thus having the largest range. That was not the case here. The poorly known factor of mortality in these species turned out to be insignificant. This is not due to the model being insufficiently refined and insensitive to the factors of mortality, as mortality was a significant factor in a sensitivity analysis run for control on the deer case (data not shown). If the model accurately reflects reality, a ban on hunting of grey ducks would therefore be unlikely to either increase the number of pure grey ducks, nor in

reducing hybridisation rates. This also means that a potential higher mortality of mallards in Australia's arid climate is unlikely to prevent widespread hybridisation.

In contrast, differences in reproductive output of either species appeared to have an effect, as did the degree of assortativeness in mating. Mallards usually lay more eggs than grey ducks, even though clutch sizes vary between locations [Rohwer 1992, Ball et al. 2002]. It is conceivable that mallard clutches are smaller under Australian conditions, while grey ducks might be less affected even in extreme conditions. However, within the ranges of mean clutch sizes used here, which span most values quoted in literature for either species, this is likely to have a limited effect only, though it might influence how much grey duck genetic material remains in a hybrid swarm. Likewise, if mating patterns differ in some populations, this could increase or decrease hybridisation. Such differences are however, rather unlikely, and if they exist the effects are likely to be limited.

Conclusion and consequences for conservation management

Studies into local reproductive output of both species and on the mating preferences of both species and of the hybrids might help more accurately assess the local risk of hybridisation for each location. However, unless they are of an extreme nature, the outcome of this simulation suggests that eventually, widespread hybridisation is very likely to ensue. It is unlikely that there are locations where conditions are such that grey duck and mallard will coexist for prolonged periods without forming a hybrid swarm. From a conservation perspective, my model suggests hybridisation is almost always an inevitable outcome of sympatry between mallards and grey ducks and that the only way

to ensure the conservation of pure grey ducks may be through their conservation in isolated allopatry.

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Chapter 7

Does inbreeding increase hybridisation rates?

A test in *Drosophila*

Abstract

Theory predicts that the relative cost of hybridisation decreases as the cost of inbreeding climbs in a population. I tested whether the propensity for interspecific hybridisation in fruit flies was higher between inbred populations of *Drosophila mojavensis* and *D. arizonae,* than between non-inbred *D. mojavensis* and *D. arizonae.* Mate choice trials were used to compare the courtship and copulation behaviour of two inbred strains of *D. mojavensis* and a wild type strain of *D. mojavensis*, when offered partners of their own strain or an outbred strain of *D. arizonae*. As found in other inbred strains, mating success was reduced for conspecific courtships in both inbred populations of *Drosophila*, but heterospecific mating success was significantly higher in one of two inbred strains when flies were offered both partners of their own line and those of another species. These results indicate that inbreeding can lead to an increased number of heterospecific matings, and highlights the role of inbreeding as a potential cause of increased hybridisation in inbred populations of endangered species.

Introduction

Hybridisation has historically been regarded as rare in animals, and restricted to populations not sufficiently divergent to reach reproductive isolation. Thus, hybridisation was seen as reducing genetic diversity, and counteracting the evolution of new adaptations, lineages or species [see e.g. Mayr 1970]. More recent evidence suggests hybridisation is a common evolutionary process of importance in animals, and happens even between well-defined species without leading to species breakdown, and indeed can lead to increased rates of evolution [Grant and Grant 1992, Arnold 1997, Dowling and Secor 1997, Schiltzhuizen 2001, Mallet 2005]. Interspecific hybridisation can lead to introgression of genetic material between species, increasing genetic diversity within a receiving population, and result in the rapid acquisition of novel alleles, genes, and the adaptations they confer.

An early example of the role of interspecific hybridisation in introgression and a resulting rapid change in fitness was discovered following the introduction of an aphid *(Dacus neohumeralis)* to Australia [Lewontin and Birch 1966]. Following the transfer of genetic material to the native *D. tyroni* by hybridisation, the latter species spread quickly into new habitats. This gain of fitness occurred despite the hybrids themselves being relatively unfit [Lewontin and Birch 1966]. A more recent example is the acquisition of novel colour patterns in African cichlids. Not only have new colour patterns been transferred by hybridisation, but this appears to happen regularly and may explain rapid speciation in this group of fish [Smith and Kornfield 2002, Smith et al. 2003, Streelman et al. 2004]. Even a probable case of hybrid speciation has been reported for the cichlid *Neolamprologus marunguensis* [Salzburger et al. 2002].

While hybridisation can sometimes confer fitness increases, the genetic effects of hybridisation can also be negative. For example, outbreeding depression can occur if co-adapted gene complexes in each parent species are broken up, or hybrids are intermediate in adaptations and suited to none of the ecological niches occupied by the parental species [Soulé 1986, Rhymer and Simberloff 1996]. Whether hybridisation leads to fitness increases or fitness decreases can be highly variable both between and within species pairs [Arnold et al. 2001, Burke and Arnold 2001, Levin 2002].

Inbred populations, in contrast, often suffer low levels of genetic diversity and high levels of homozygosity, in some cases reducing the viability of individuals and the population. Inbred populations are predicted to suffer detrimental effects such as high susceptibility to disease and parasites, and a reduced adaptability to changing environments [Lacy 1997]. In extreme cases, inbreeding depression can result, with effects including reduced viability, growth rates, fecundity or physiological efficiency [Lande 1988, Mitten 1993] and even increased extinction risk [e.g. Petterson 1985, Wayne et al. 1991, Mlot 1993, Frankham 1995, Laikre et al. 1997].

The negative effects of both inbreeding and outbreeding depression might in cases partially counteract each other in hybridisation between two previously isolated populations. It is possible that introgression of foreign genes could sometimes lead to a genetic rescue [Tallmon et al. 2004]. While a negative effect might be at least as likely as a positive one, a population under strong pressure might profit from a “genetic gamble” from hybridisation. This is less controversial and better supported for intraspecific than interspecific hybridisation [Edmands 2007], but theoretically could apply to both.

In a population suffering from inbreeding depression, for example, and no access to non-inbred mates of the same species, interspecific hybridisation may be one viable way for an individual to acquire genetic diversity for its offspring, and thus reduce the cost of inbreeding depression. It is expected that the relative cost of hybridisation should decrease as the cost of inbreeding depression increases [Burke and Arnold 2001]. Thus, the more inbred a population becomes, the less damaging, or even the more advantageous hybridisation may become. It might therefore be advantageous for individuals in inbred populations to engage in hybridisation more frequently than individuals in non-inbred populations of the same species.

Such a predicted effect of hybridisation on an inbred population has been demonstrated in two species of Darwin's finches on the Galapagos (*Geospiza fortis* and *G. scandens*) [Grant et al. 2003, Grant et al. 2004]. The relative fitness of hybrid backcrosses of *Geospiza scandens* is higher than that of inbred individuals of the same population, but hybridisation holds no advantages for the less inbred *G. fortis* [Grant et al. 2003, Grant et al. 2004]. Gene flow from *G. fortis* to *G. scandens* has been confirmed by molecular methods, and has been related to a change in beak size, which leads to a rapid adaptation to the changing environment [Grant et al. 2004]. In fact, the advantage of hybrids is environmentally dependent in this case [Grant 2003]. A similar example of hybrid advantage is the endangered and inbred Forbes parakeet (*Cyanoramphus forbesi)*, in which hybridisation with a more common species increases immunity responses [Tompkins et al. 2006].

It has been suggested that hybridisation as a mechanism for greater adaptability might be most common when changes in the environment or disturbance rates are high [Pierotti

and Annett 1993]. Hybrid advantage might therefore be expressed only in particularly stressful conditions. If this is generally the case, then hybridisation and introgression should be seen at increased rates in populations under stress due to habitat changes or inbreeding depression. It also means that an absence of hybrid advantage in one sample and situation cannot be taken as evidence that there might not be an adaptive advantage to hybridisation if conditions change. Hybridisation might even, in species that experience regular environmental changes or genetic stresses, be a facultative response to such situations.

Even in the absence of an adaptive advantage, hybridisation rates may be increased if inbreeding avoidance mechanisms are more developed than outbreeding avoidance mechanisms. This has been shown in the copepod *Tigriopus californicus* [Palmer and Edmands 2000]. Female copepods avoid inbreeding with siblings but do not avoid outbreeding despite larger fitness losses – possibly there has been no historical selection pressure to develop outbreeding avoidance, since populations do not normally come into contact.

If inbreeding does heighten the rates of hybridisation, this could help explain why small populations are at a higher risk from hybridisation than larger ones. This has traditionally been attributed to the rarity of mating partners (a heterospecific partner is selected out of desperation), and this is indeed a likely cause in many endangered species [Mayr 1970, pp 326-327, assumed a problem for rare species in Rhymer and Simberloff 1996, Randler 2002]. However, since endangered populations are also frequently inbred, it could also be a lack of mating partners with suitable genetic variation that increases hybridisation risk.

Many *Drosophila* species are known to hybridise [Bock 1984], and in some cases inbreeding avoidance has been demonstrated – both inbred *D. melanogaster* and inbred *D. montana* avoid partners of their own lines in preference to those of other, equally inbred lines [Averhoff and Richardson 1973, Averhoff and Richardson 1975, Markow 1982, Suvanto et al. 2000]. However, a number of other studies found no such preference [see Spiess 1987, Suvanto et al. 2000].

The cactophilic *Drosophila* species *D. mojavensis* and *D. arizonae* hybridise in the laboratory even if conspecific partners are available, but do not do so indiscriminately. Allopatric strains are more likely to hybridise than sympatric ones [Markow 1981, Massie and Markow 2005]. Wild *D. mojavensis* and *D. arizonae* share no mitochondrial haplotypes, so despite sympatry and ease of hybridisation in the laboratory, there is no evidence of recent introgression [Counterman and Noor 2006, Reed et al. 2007]. Estimated divergence time is 1.91 to 2.97 million years ago [Reed et al. 2007].

In this chapter, I used these two species to experimentally test for the propensity of inbred and non-inbred *D. mojavensis* to mate with *D. arizonae*. My expectation was that a simulated population of inbred *D. mojavensis* in contact with *D. arizonae* would have a higher rate of heterospecific copulations than one of non-inbred *D. mojavensis* in the same situation. Two different inbred lines were used since different genes may be fixed in different inbred lines, thus potentially impairing their mating behaviour. Little conclusions can therefore be drawn if only one inbred strain is used. To further investigate whether any differences were due to overall low mating success of inbred individuals – for example due to impaired courtship ability or attractiveness – or if individuals preferred genetically dissimilar individuals, I also tested inbred *D. mojavensis*

when given a choice of *D. arizonae* and *D. mojavensis* of another inbred strain. Should inbred flies *per se* hybridise more than non-inbred flies, no matter what type of conspecific partner was available, this might suggest that higher hybridisation rates are due to an impaired mate-choice or courtship ability of inbred flies. If, on the other hand, levels of hybridisation should be lower if inbred flies of another line were offered, hybridisation might rise because inbred flies select against partners genetically very similar to themselves, but not inbred individuals *per se*.

I tested both male and female choice, since both male interest and female acceptance are critical to potential hybridisation. In *Drosophila*, males as well as females are known to discriminate in mate choice (see for example Byrne and Rice 2006). If males do not court females of another species in the first place, hybridisation is impossible. If females do not accept any courtships, hybridisation is again impossible. Investigating the behaviour of both sexes gives a better indication of preference mechanisms than observing only one sex.

If inbreeding plays a role in interspecific hybridisation, I expected the following: (1) inbred flies would copulate with or court a heterospecific partner more often than wild type flies, (2) inbred flies would copulate with or court a heterospecific partner more often if offered a partner of their own line than if offered a partner of another inbred line, (3) the success of conspecific courtships would be higher in wild type than in inbred trials, (4) the success of heterospecific courtships should be the same across all trials, (5) courtship duration should be longer for heterospecific than conspecific copulations, (6) conspecific courtships should last longer in inbred than wild type pairings, and (7) heterospecific courtships should be shorter in inbred than wild type pairings.

Methods

Drosophila stocks

Two laboratory stocks of *Drosophila mojavensis*, each originating from a single female collected on Catalina Island, California (*D. mojavensis* CI10-IB-B6 and *D. mojavensis* CI12-IB4) were used as inbred experimental populations. More than two strains would have been desirable, but were unavailable during the time of study. The offspring of four freshly collected, gravid female *D. mojavensis* from Catalina Island were mixed, and the resulting offspring served as a wild type, non-inbred control. The *Drosophila arizonae* were of the TU702 strain, a non-inbred strain originating from the Tucson area in Arizona. *D. mojavensis* from Catalina Island are particularly likely to hybridise with *D. arizonae* [Massie and Markow 2005]. Hereafter, the strains of *D. mojavensis* are referred to as Inbred1 (Ci10-IB-B6), Inbred2 (Ci12-IB4), and WT (non-inbred wild type flies).

Culture of flies

Flies were cultured in standard half-pint milk bottles and fed on banana *Opuntia* medium with yeast supplied by the Tucson *Drosophila* Stock centre. Tissue paper was supplied to allow the flies to perch and pupate. Bottles were purged and virgin flies were collected daily. The flies were sexed under CO_2 anaesthesia and single-sex groups of 6 to 10 individuals were placed for 11 days in vials with cornmeal/yeast medium before use in mating experiments. Flies were transferred to fresh medium half-way through this 11 day period.

Mate choice trials

Mate choice trials were conducted in Plexiglas chambers measuring approximately 12 cm diameter and 1.8 cm height. The day before the trial, flies were coloured with fluorescent powder (Radiant Corp., Richmond, CA) to enable identification. For a trial testing male mating preference, *D. arizonae* and *D. mojavensis* females were coloured either red or blue; for trials testing female mating preference, the males were coloured in the same fashion. Colouring was done the day before the trial to enable the flies to clean off excess powder. Colours were swapped between species and between trials to control for a possible effect of colour on mate choice. Experiments were conducted from 0700 to 0900 MST, which is the period of peak activity for mating in these species. Flies were stored in a dark place until use.

All mate choice trials had the following design. In male mate-choice trials, five *D. mojavensis* females and five *D. arizonae* females were gently aspirated into the mating chamber, and then ten *D. mojavensis* males were added. In female mate-choice trials, five *D. mojavensis* males and five *D. arizonae* males were first introduced, and then ten female *D. mojavensis* were introduced into the chamber. Thus, the sex ratio was even in each trial (10 males and 10 females) but the individuals of the observed sex had the option of mating with either a member of their own species or that of the second species. The ratio of the two species available for selection was also even (5 of each species) and so the choice made by individuals of the observed sex was not confounded by the relative abundance of the two species.

The inbreeding status of the males and females was varied between trials such that five sets of mate choice trials were run. The first set of trials tested the hybridisation

likelihood of WT *D. mojavensis* when given a choice of WT partners or *D. arizonae*. Two further trials tested the hybridisation likelihood of each inbred line when offered inbred partners of their own lines or *D. arizonae* (inbred1 x inbred1 and inbred2 x inbred2). The last two trials tested the hybridisation likelihood of either inbred strain if given a choice between the other inbred line or *D. arizonae* (inbred1 x inbred2 and inbred2 x inbred1). Between 15 and 36 replicates of each trial were conducted.

Mating trials lasted one hour, and observation began as soon as all flies were introduced into the chamber. Up to three mating chambers were watched at a time. The starting time of any courtship and whether it had involved a *D. arizonae* or *D. mojavensis* fly, the duration of courtship, and start and end time of any copulation were then recorded to the nearest minute, or if shorter than a minute to the nearest 10 seconds.

For the analysis of courtship duration in female mate-choice trials, all successful courtships (i.e., those resulting in copulation) were included in the analysis. For male choice trials only unsuccessful courtships were used. Unsuccessful courtships are not cut short by copulations, so are a better measure of how much time a male is prepared to invest in a given female. However, the dataset for males was too small for several trials to be reliably analysed, therefore only data for females are presented.

Statistical analyses

Each trial was treated as a single data point to determine proportions of heterospecific to conspecific courtships and copulations, as well as the success rate of conspecific and heterospecific courtships. Trials in which no copulations at all were observed were excluded where appropriate (56 out of a total of 267 trials). Copulations lasting under 100 seconds were assumed to be pseudo-copulations, in which no sperm is transferred, and therefore disregarded in the analysis. No safe criteria for the identification of pseudocopulations are available, other than the deposit of sperm. The 100 seconds chosen is somewhat arbitrary, but likely to err on the side of caution.

The Empirical Logit Transformation was used to calculate proportion values. For this transformation, a small constant is added and then the ratios and logarithms calculated (Siegel 1988, McCullagh and Nelder 1989). The exact transformations are given below.

For proportions:

log((heterospecific copulations+0.5)/(conspecific copulations+0.5))

For success rate:

log((copulations+0.5)/(courtships+0.5))

This procedure avoids zero denominators, and thus a ratio being undefined, and numerators of zero that lead to a ratio of zero regardless of the denominator. It also takes into account total numbers and not just raw proportions. For example, a record of no copulations in an entry of one courtship is treated differently from one of no copulations

in an entry of fifteen, while both would have a simple success ratio of zero. As this transformation leads to a normal distribution of error, the data were analysed by a General Linear Model. An exception was the courtship values, for which a square root transformation was needed to normalize the data. With this modification, all four values conformed with the assumptions of the General Linear Model. The corresponding back-transformation is 10**y/(1+10**y), and gives the proportion or success rate. All values quoted in the results section have been back-transformed.

General Linear Models tested for effects of the cross type, taking into consideration the test sex and any interactions between them where appropriate. This was the case when testing for proportion of heterospecific courtships, but not copulations where only females were analysed due to a lack of data in males, and when analysing the success of heterospecific and conspecific courtships. Pairwise comparisons after Tukey were performed, and were the main statistic for courtship durations. The correction after Tukey makes false positives less likely in view of the large number of tests. All statistics were performed in Minitab (version 15.1.0.0).

Results

Data summary

All inbred, and in particular inbred1, individuals were extremely reluctant to mate. In 58 trials, involving 580 inbred1 females, only 68 copulations were observed, 5 of which were with *D. arizonae* males. More data were available for the inbred2 females. In 59 trails (590 females), 125 copulations took place, of which 67 were with *D. arizonae* males. In the wild type, for comparison, 32 trials (320 females) resulted in 231

copulations of which 23 with *D. arizonae* males. This strong asymmetry led to insufficient data to reliably test for some relationships in this data set, especially for male choice.

Table 1. Mate choice by male *D. mojavensis* when presented with both conspecific females and heterospecific *D. arizonae*. Figures are average number/one hour trial (± Standard Error). N is the number of replicate trials. Ten *D. mojavensis* males, five D. mojavensis females and five *D. arizonae* females (not listed in table) in each trial. Copulations are therefore out of a possible five each.

Male x female	N	Conspecific courtships	Heterospecific courtships	Conspecific copulations	Heterospecific copulations
WT x WT	30	19.3 ± 1.4	13.8 ± 1.5	4.0 ± 0.2	0.9 ± 0.2
Inbred1 x inbred1	20	8.1 ± 1.0	6.6 ± 0.9	0.4 ± 0.2	0.0 ± 0.0
Inbred1 x inbred2	20	16.3 ± 2.0	5.2 ± 1.0	1.0 ± 0.3	0.05 ± 0.05
Inbred2 x inbred2	27	28.4 ± 2.2	16.4 ± 1.6	2.5 ± 0.3	0.1 ± 0.1
Inbred2 x inbred1	22	26.9 ± 2.9	10.3 ± 1.2	1.6 ± 0.3	0.04 ± 0.04

I found differences between the lines in courtship and copulation behaviour (Table 1). Inbred1 males showed low courting activity compared to WT males, especially towards their own females (Table 1). Inbred2 males, in contrast, courted exceptionally frequently. The copulation success of males of all inbred lines was also lower than of wild type males, but more dramatically for inbred1 males than inbred2 males.

Table 2. Mate choice in female *D. mojavensis*. Table shows averages per trial. Ten *D. mojavensis* females, five *D. mojavensis* males and five *D. arizonae* males (not listed in table) in each trial. Copulations are therefore out of a possible ten each.

Female x male	N	Conspecific courtships	Heterospecific courtships	Conspecific copulations	Heterospecific copulations
WT x WT	32	19.9 ± 1.1	11.1 ± 1.1	6.5 ± 0.3	0.7 ± 0.2
Inbred1 x inbred1	36	13.1 ± 2.0	5.9 ± 0.5	1.2 ± 0.2	0.1 ± 0.1
Inbred1 x inbred2	15	24.7 ± 2.5	4.7 ± 1.1	2.9 ± 0.5	0.2 ± 0.1
Inbred2 x inbred2	32	22.6 ± 1.6	8.6 ± 1.2	1.7 ± 0.3	1.0 ± 0.3
Inbred2 x inbred1	24	17.1 ± 1.8	10.2 ± 2.0	1.7 ± 0.3	1.2 ± 0.3

Inbred females had much lower numbers of copulations than wild type females (Table 2). However, I did observe a relatively high number of inbred2 females copulating with *D. arizonae* males (Table 2). There was again a higher number of courtships towards conspecific females in inbred2 strain males, and a low likelihood of *D. arizonae* males courting inbred1 females (Table 2). Male *D. arizonae* did rarely court inbred1 females even if inbred2 males were present, which court inbred1 females readily. It therefore appears that *D. arizonae* males did not consider inbred1 females attractive, independent of the stimulus of other males courting.

Proportion of heterospecific copulations

The analysis of female choice revealed that the proportion of heterospecific copulations was significantly influenced by the type of trial (GLM proportion = trial type, $p<0.001$; r^2 adjusted = 26.27%) (Figure 1). Inbred2 females had significantly higher rates of heterospecific copulation than the WT control (pairwise comparison, $p < 0.001$), while differences for inbred1 females were not significant (pairwise comparison, $p > 0.05$).

I found that 12.4% of all copulations of WT females were with *D. arizonae* males, and 17.7% of all copulations of WT males were with *D. arizonae* females. However, inbred2 females copulated with *D. arizonae* males in 37.2% of cases if also offered inbred2 males, and 46.7% of cases if also offered inbred1 males. These two trials did not differ significantly from each other (pairwise comparison, $p > 0.05$), so if offered males from their own strain, inbred1 females were not more likely to hybridise than if offered males from another, equally inbred strain. An insufficient number of heterospecific copulations were observed in trials involving inbred flies for reliable analysis of male choice.

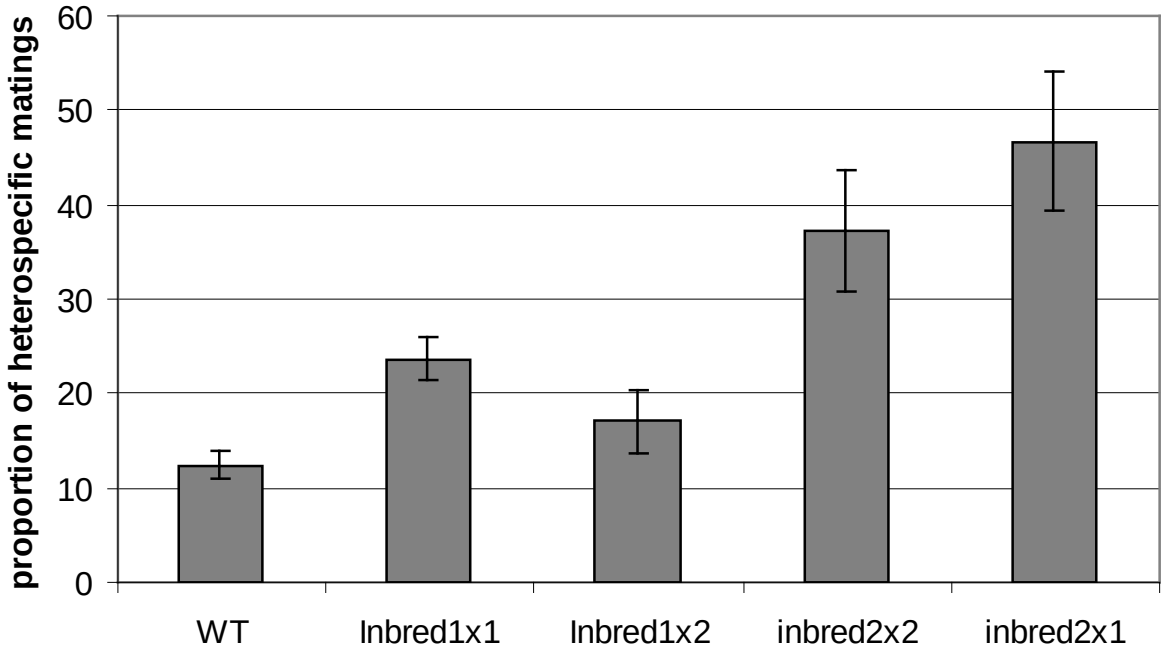

Figure 1. Proportion of copulations of female *D. mojavensis* with *D. arizonae* males in five different trials when offered a choice of males of both species. WT – 10 wild type *D. mojavensis* females and 5 wild type *D. mojavensis* males (plus 5 male *D. arizonae*), otherwise female line x male line of *D. mojavensis* (plus 5 male *D. arizonae*).

Proportion of heterospecific courtships

WT males courted *D. arizonae* females on an average of 27.6% of all courtships, the remainder of courtships being aimed at WT females. On average, one quarter (24.5%) of courtships experienced by WT females were from *D. arizonae* males.

The proportion of total courtships aimed at a heterospecific partner was significantly influenced by the test sex and the type of trial due to a significant interaction between these two terms (GLM proportion = sex + trial type + sex*trial type, sex $p = 0.320$; trial type $p = 0.244$; interaction $p < 0.001$; $r^2 = 8.33\%$). However, due to the low r^2, and no

pairwise comparisons being significant, it was assumed that the effect was unlikely to be of biological importance, and no further results are presented.

Relative success of conspecific courtship

Courtships between inbred *D. mojavensis* were less likely to result in copulation than courtships between wild type *D. mojavensis* (GLM success = sex + trial type + sex*trial type) $p < 0.001$, sex $p = 0.002$; interaction of both terms $p = 0.014$; adjusted $r^2 = 37.13\%$) (Figure 2). A total of 19.2% of all courtships between WT flies in male choice trials and 25.9% in female choice trials lead to copulations. In contrast, only between 6.0% and 11.4% of courtships between inbred flies, no matter if of the same or different strain, lead to copulations. In pairwise comparisons, all four inbred trials differed from the WT trials in either sex ($p < 0.001$).

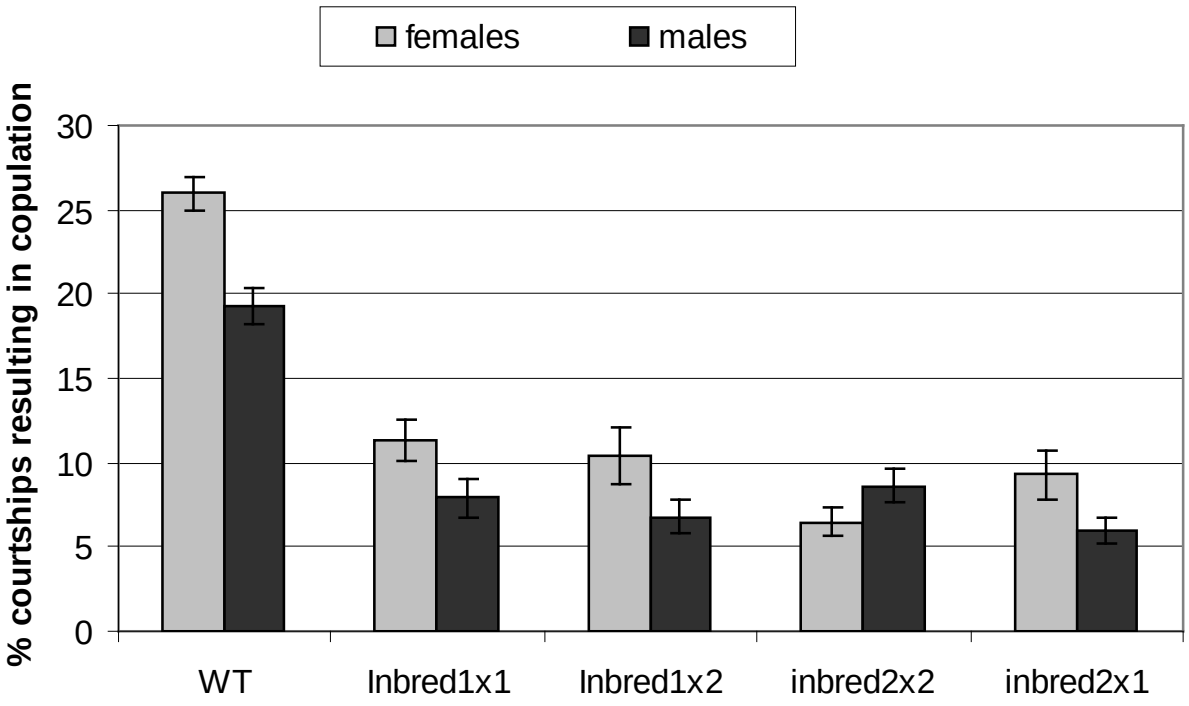

Figure 2. Proportion (in percent) of courtships between conspecific *D. mojavensis* resulting in copulations. WT – 10 wild type individuals of the target sex, and 5 wild type individuals of the other sex of *D. mojavensis* (plus 5 non-target sex *D. arizonae*), otherwise target sex line x non-target sex line of *D. mojavensis*

Relative success of heterospecific courtships

While inbred flies were more reluctant to mate with conspecifics, they were not more reluctant to mate with heterospecific individuals (GLM proportion = sex + trial type + sex*trial type, all $p > 0.05$), excepting one of eight cases (pairwise comparison, male inbred2x2 differed significantly from the WT $p < 0.001$) (Figure 3). A total of 8.5% of *D. arizonae* courtships were accepted by WT females, and 9.1% of attempts by WT males to court *D. arizonae* females were successful. Note that despite females of the inbred1 line being very rarely courted by *D. arizonae* males, the likelihood of those rare courtships

being successful was not diminished. In the inbred2 line, the females in particular drove this trend to higher hybridisation, while males were less likely to be accepted by *D. arizonae* females (pairwise comparisons, $p < 0.001$).

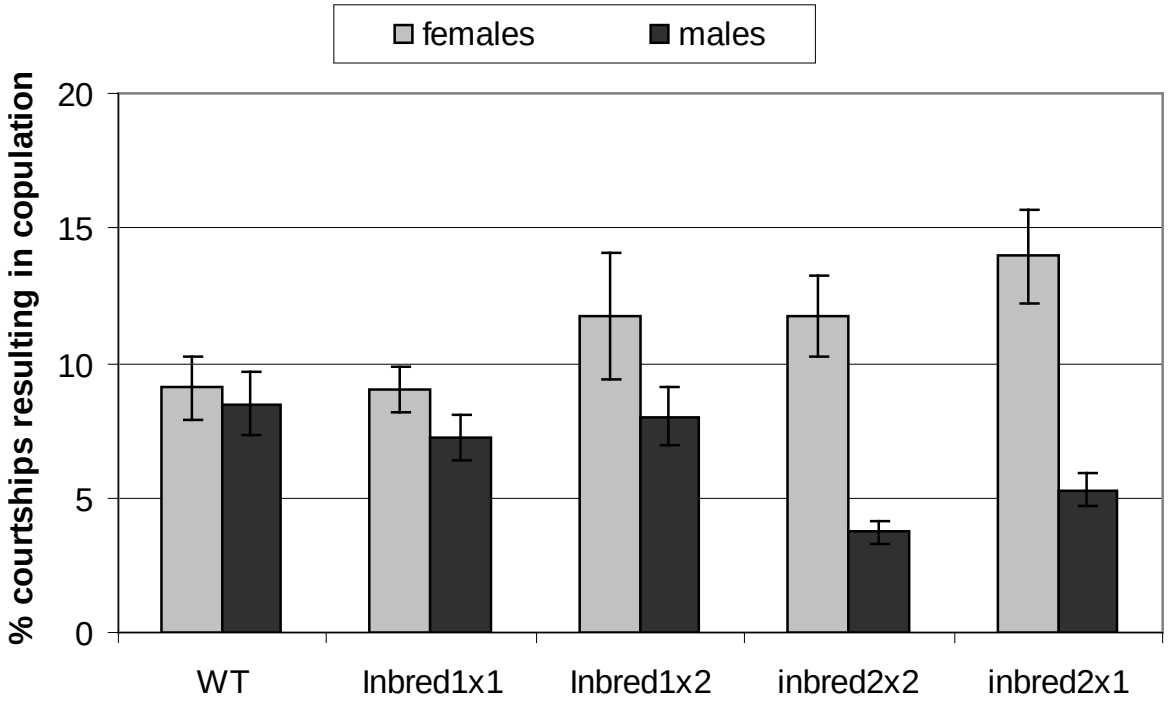

Figure 3. Proportion (in percent) of courtships between heterospecific *D. mojavensis* and *D. arizonae* resulting in copulations. WT – 10 wild type individuals of the target sex, and 5 wild type individuals of the other sex of *D. mojavensis* (plus 5 non-target sex *D. arizonae*), otherwise target sex line x non-target sex line of *D. mojavensis.*

Courtship duration

To copulate with an inbred female, conspecific males had to invest in longer courtships than heterospecific males (Figure 4), while no such difference existed for wild type females. Conspecific courtships involving inbred flies were significantly longer in

three out of the four trials than courtships between wild type flies (pairwise comparison, $p > 0.001$ to 0.002 in inbred1x1, inbred1x2 and inbred2x2, but $p = 0.141$ in inbred2x1). In contrast, no significant difference existed between heterospecific courtship duration of any of the five trials (pairwise comparison, all $p > 0.05$), and heterospecific courtships and conspecific courtships accepted by WT females did not differ significantly in duration (pairwise comparison, $p = 0.266$). In all but one trial involving inbred flies heterospecific courtships were significantly shorter than conspecific ones (pairwise comparisons, $p = 0.034$ in inbred1x2, and 0.000 in inbred2x2 and inbred2x2, but inbred1x1 $p = 0.216$). The lack of significance in inbred1 x inbred1 may be due to the low overall number of copulations, leading to a lack of confidence in the estimates.

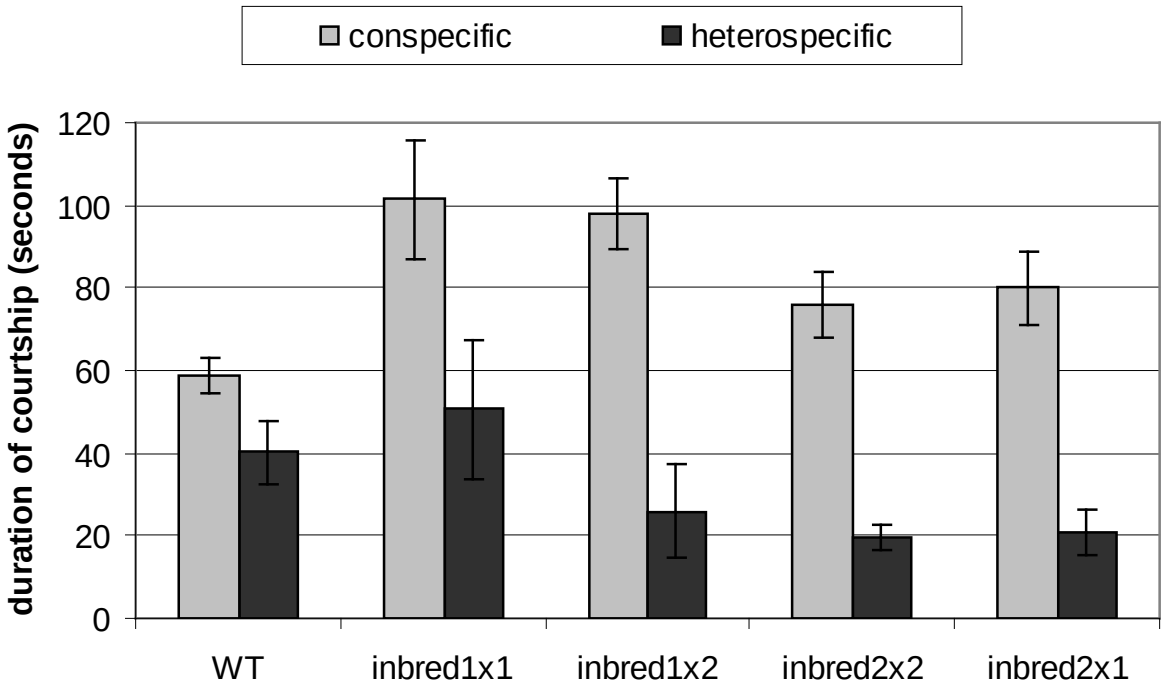

Figure 4. Average duration in seconds of conspecific and heterospecific courtships for female *D. mojavensis* presented with a choice of *D. mojavensis* males and *D. arizonae* males. Courtships resulting in copulation only. WT – 10 wild type *D. mojavensis* females, and 5 wild type *D. mojavensis* males (plus 5 male *D. arizonae*), otherwise female line x male line of *D. mojavensis.*

Discussion

Summary of Results

Contrary to expectation (1), females of only one of the two inbred *D. mojavensis* lines copulated with *D. arizonae* males in a larger fraction of all matings than wild type flies, and no conclusive results can be presented for male *D. mojavensis*. However, when taking into account the reduced courtship frequencies in inbred flies by calculating the rate of courtships resulting in copulations, I found support for my prediction (3), that

conspecific success was reduced in inbred lines relative to wild type lines for both males and females, and prediction (4), that heterospecific success was unchanged.

The lower mating propensity of inbred *D. mojavensis* strains compared to wild type flies has been documented previously [Markow 1982]. Inbred *Drosophila* males appear less able to compete for partners, often displaying atypical courtship song and behaviour [Sharp 1984, Suvanto et al. 2000]. It was therefore not the reduced rate of conspecific courtship success that was surprising, but rather that the rate of heterospecific success was unaltered. Inbred *D. mojavensis* appeared to discriminate against inbred partners, no matter if closely related or not, but they were just as likely to mate with a *D. arizonae* as wild type *D. mojavensis* were, provided they were being courted or accepted. This had the net effect of increasing the rate of heterospecific copulations. It also shows that inbred individuals were not, *per se*, unwilling to mate.

It appears puzzling that this difference in success rates leads to significantly more interspecific copulations with *D. arizonae* compared to the wild type line only in one of the two inbred strains of *D. mojavensis* studied, while the difference was not significant in the other line. On closer inspection, this appears to be due to the fact that *D. arizonae* males rarely courted the females of that line (inbred1) under any conditions. The few courtships that did occur, however, were still accepted at a high rate. In this case, *D. arizonae* males appeared unwilling to mate, while the inbred *D. mojavensis* females were willing to hybridise. However, contrary to my prediction (2), no significant differences were found between inbred *D. mojavensis* that were offered partners of their own line and those offered partners of another inbred line. It therefore appears that it is not the case

that flies select partners dissimilar from themselves, and instead might have a preference for genetically diverse, non-inbred partners.

Interestingly, and contrary to my prediction (5), the duration of courtships was shorter for heterospecific than for conspecific trials. This suggests that females, inbred or not, mate with heterospecific males either after a short courtship or not at all, but long courtships are very rare. As expected (prediction 6), conspecific courtships lasted longer in trials using inbred flies than in those using wild type flies, but unexpectedly (prediction 7) heterospecific courtships were not shorter in inbred trials, but the same as in wild type trials. Thus inbred flies did not accept heterospecific partners any faster than wild type flies, but they were slower to accept inbred males.

Differences between inbred lines

Differences between inbred lines are to be expected since different alleles will become fixed in different lines. This was the predominant reason for including more than one line in my study. For example, inbred lines of *Drosophila melanogaster* usually show impaired male mating behaviour, but the way in which it is impaired can differ between lines [Miller et al. 1993, Miller and Hedrick 1993, Suvanto et al. 2000]. As my study shows, inbred females can also be less attractive to males, though interestingly more so to males of another species than those of their own. Both males and females of the inbred1 line appear behaviourally impaired, and most notably they are remarkably inactive, as the low number of courtships observed per trial shows.

No preference of other inbred line over own line

Neither of the two strains studied showed a preference for mates of the other inbred line over those of their own. This was expected, as it was thought that the flies might practice classic inbreeding avoidance as once described for *D. melanogaster* by Averhoff and Richardson [1973]. However, the results of that paper have not been reproduced, with further studies showing no such tendencies when attempting similar experimental designs [see Spiess 1987, Suvanto et al. 2000]. It is therefore doubtful that *D. melanogaster*, and possibly other *Drosophila* spp, exhibit classic inbreeding avoidance by choosing partners dissimilar to themselves even if they are likewise inbred. Here, I find that instead, flies discriminate against any inbred partners, disregarding the degree of similarity to themselves. The reason is not clear, and further studies would be required to understand it. There are, however, two immediately apparent explanations that might warrant testing. The first is simply that low general activity levels and an inability to perform normal courtship behaviour in inbred flies (see above) make them unattractive and undesirable partners, or unable to choose properly. Different lines might be impaired in different ways, but might still be impaired to a similar degree. Indeed in inbred trials, the proportion of heterospecific and conspecific courtships that resulted in copulation were similar, while they clearly differed for wild type flies. However, the difference in courtship length required for a successful copulation between heterospecific and conspecific pairings may suggest that the females, at least, are capable of distinguishing between the males of either species (but see below for a more critical discussion of this point). The second possibility is that flies might not exert avoidance of partners similar to themselves, but select genetically diverse partners irrespective of the degree of similarity

to themselves. Heterozygosity has been shown to be a desired attribute in partners of several species, but not in others, probably because individuals with high heterozygosity are healthier and more attractive in these cases [Brown 1997, Roberts et al. 2006].

Courtship duration

Interestingly, I found that courtships preceding heterospecific matings were of a shorter duration than those preceding conspecific matings. It had been expected that females would be more reluctant to accept a partner not of their own species, and thus that courtships would last longer. To the best of my knowledge, this behaviour has not been documented, or studied, before. It appears that females either reject a heterospecific male, or accept it quickly. This was true independent of the individuals' inbreeding status. In fact, the length of courtships between heterospecific flies remained the same length, independent of inbreeding status, while courtships between inbred conspecific flies take longer than those between wild type flies. The equal length of heterospecific courtships in inbred and wild-type flies was entirely unpredicted.

Two likely explanations come to mind. The first is that females simply commit a mistake when choosing a male of another species, and that a longer courtship makes them aware of this mistake and therefore prevents copulation. This, together with a less pronounced ability of inbred individuals to discriminate between partners of their own and another species, could in fact explain much of the patterns observed in this study. Alternatively, the equal length of heterospecific courtships might be due to the fact that females recognise a male from a different species immediately, but make a fast choice to mate them regardless – possibly, courtships are short because females seek no other

message after they have identified a partner as being of another species, and in the case of inbred flies, as not closely related to themselves. The first explanation appears more likely at first glance, but further work will be required before a conclusion can be drawn.

Limitations of this study: further desired trials

Trials of a third and fourth type, giving inbred flies a choice of wild type partners or *D. arizonae*, and giving wild type flies a choice of inbred partners or *D. arizonae*, would have helped to clear some of the questions raised above. Unfortunately, given constraints of time and material, these trials could not be conducted within the limits of this study. It also seemed wasteful to plan this many types of trial for a first investigation of the topic. The results found here might warrant such studies in the future, and also the inclusion of additional inbred lines.

Limitations of this study: post-mating isolating mechanisms

While this present study shows a tendency for increased rates of interspecific copulations due to inbreeding, it investigates mating preferences only, not taking into account any post-mating isolation mechanisms. Higher rates of interspecific copulations do not have to result in an increase in hybrid offspring. Instead, it is possible that post-mating isolation and cryptic female choice could reduce the number of hybrid offspring to the same level as in non-inbred populations, or even lower. This could happen if, for example, fewer sperm are transmitted in some types of crosses than in others, and if less of it was used to fertilize eggs. This has previously been shown between members of the *D. virilis* group [Markow 1997], and between *D. athabasca* and *D. affinis* [Miller 1950],

where females contain fewer and less motile sperm after heterospecific crosses than after conspecific copulations. In *D. simulans*, *D. sechellia* and *D. mauritiana*, heterospecific sperm fertilises fewer eggs than conspecific sperm no matter the order of the two copulations [Price 1997]. The exact mechanisms of post-mating isolation vary between these species pairs. They include shortened copulations, little sperm transfer even in long copulations, fewer sperm being stored or the sperm being lost rapidly from storage, and fewer eggs being laid [Price et al. 2001]. It is possible that such mechanisms also occur in the species pair used here. To my knowledge, this has not been investigated.

Post-mating isolation could also have the opposite effect and increase the number of hybrid offspring further, by reducing the use of sperm from inbred individuals by selective fertilisation. *D. melanogaster* [Clark et al. 1999, Mack et al. 2002] and *D. nigrospiracula* [Markow 1997] avoid the use of sperm of close relatives, even if they mate with them, and *D. mojavensis*, the species used in this study, will lay less eggs if mated to a sibling than if mated to an unrelated individual [Markow 1997]. Such avoidance of inbreeding could lead to numbers of hybrid offspring being larger than expected from the number of interspecific copulations observed. It remains for another study to show if this indeed is the case in this species pair.

In light of such opposing post-mating isolating mechanisms – hybrid avoidance and inbreeding avoidance – it is difficult to estimate the actual evolutionary effect of the increased hybridisation rates observed here. Unfortunately, it was outside the scope of this study to investigate this important and interesting point. It would be highly desirable for such work to be done in the future, by isolating mated females and allowing them to breed. Counts of eggs, and larvae, should give a better indication of the rates of hybrid

offspring. Besides counting the offspring of those females mated conspecifically and heterospecifically, it could further be investigated what happens in the case of females that mate with partners of both species. Here, cryptic female choice might be particularly relevant. However, such a study would be complicated by the fact that the only reliable way to identify hybrid offspring between the two species is probably by molecular methods.

Conclusion and implications

Despite these caveats, my results indicate that at least in some cases, inbreeding can lead to an increased number of heterospecific copulations. Further studies will be needed to confirm the results presented here, and to show that an actual increase in hybrid offspring results. Ideally, studies in other species and genera would follow. It will also have to be investigated if such results are seen only in extremely inbred lines, such as used here, or also in more moderately inbred populations as are more likely to occur in the wild.

If it should turn out that, indeed, inbred populations are more prone to hybridisation than non-inbred populations, this is of potential concern to managers of small and inbred populations of endangered animals that engage in hybridisation. It would offer a new management practise for preventing hybridisation, namely to broaden the gene pool and encouraging outbreeding. This could happen by increased transfer of individuals from isolated, small populations, or by augmentation of individuals from captive breeding programs.

Such a result would however also be of interest in the light of the current debate about the adaptive value of hybridisation. If it can indeed be shown that inbred individuals are more likely to engage in hybridisation, this would lead to the question if such hybridisation are due to mistakes and impaired courtship or mate choice mechanisms in inbred individuals, or if they might be adaptive, or even a facultative adaptive response. It is possible that inbred individuals from a population with an impoverished gene pool are more likely to profit from the introduction of genes across species boundaries. While convincing in theory, it remains to be shown if this is the case in reality, at least for some organisms in some circumstances. Taken to its logical (if highly speculative) conclusion, this could indicate that some species that were historically prone to the formation of isolated, inbred populations might even seek hybridisation in such circumstances as a facultative adaptive response. If this were the case, this would open a new view to hybridisation in such populations, away from the hypothesis that a lack of partners leads to individuals committing mistakes, towards a view that it is an adequate and successful strategy. It remains to be seen if future studies will take up and investigate this new field of investigation.

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Chapter 8

Discussion and Conclusion

Summary of findings

Hybridisation of grey duck and mallards in New Zealand is well advanced. Grey ducks are rare even where they are most common, and they are declining, while hybrids make up about half of the population in most locations surveyed and seem to be increasing. Both grey ducks and mallards mate assortatively, choosing partners of their own species. However, hybrids (that occur by whatever mechanism) generally mate with other hybrids, if by preference or exclusion from other partners, and thus increase the proportion of hybrids rather than mediate limited gene flow between the species. This mating pattern is likely to have been influential in establishing the current hybrid swarm. Results from molecular analysis back up the results of my field survey. All of the ducks caught were hybrids, although two were cryptic hybrids that had a mallard phenotype, and genetic material of mallards dominated overall. The phenotype of an individual predicted its species genotype only very roughly and should not be used as a diagnostic other than at a population level. No reliable differences in health of grey-like and mallard-like hybrids were found, but no pure grey ducks and very few putative pure mallards could be included in the study. It appears that differences in health or condition with hybrid status is unlikely to be a major selection pressure for one or the other hybrid type at this point in time, although mallard-like individuals possibly are of slightly better condition than grey-like ducks. A modelling approach predicts that the two species will mix under all reasonable parameter ranges. However, the outcome of hybridisation, and particularly the amount of genetic material contributed to the final population by either parental species, is influenced by reproductive output and the level of assortative mating, but surprisingly not by relative mortality within the assumed ranges. Finally, a study

using *Drosophila* established the possibility that inbred populations might be more prone to hybridisation than non-inbred populations of the same species. Although it is unlikely that hybridisation was initially a product of inbreeding in grey ducks, the introduction of mallards to New Zealand did involve a population bottleneck that may have increased inbreeding and favoured hybridisation. However, even if this was not the case with mallards, my results suggest that increased probability of hybridisation may be more likely in other endangered animals.

The grey duck and mallard hybridisation in New Zealand: a historical perspective

It appears that hybridisation between grey duck and mallard in New Zealand was limited at first after the introduction of the mallard. Some hybrid individuals were noticed early [Knox 1969, Marchant and Higgins 1990], but it became a widespread phenomenon only later [Knox 1969, Gillespie 1985, Hitchmough et al. 1990]. This pattern can be explained by the assortative nature of mating, and indeed it was predicted by the mathematical model. The mechanism by which these first cases of hybridisation arose is unknown, and it might have been due to mispairings, and forced copulation, but there could also have been cases of nest amalgamation followed by imprinting [Randler 2002]. Both species are known to engage in egg dumping [Marchant and Higgins 1990, Randler 2005], a behaviour in which females lay eggs in the nests of other females. As the males of *Anas* spp. imprint on their mothers as a model of further mating partners [Bauer and von Blotzheim 1968, Williams 1983, ten Cate and Vos 1999], such ducks might be imprinted on females of the wrong species. Male mallards also routinely engage in forced copulations [McKinney et al. 1983, Seymour 1990, Randler 2005], and are known to be

willing to mate with females of other species [see e.g. Seymour 1990]. With the data available it is impossible to determine which, or which mixture, of these mechanisms were responsible, and it is likewise unknown what role they play in maintaining the current hybrid swarm.

By whatever mechanism first hybrids appeared, if they could find other hybrids it is likely that they mated selectively with each other, thus quickly increasing the hybrid proportion of the population. Backcrosses of hybrids with either parental species would then further threaten the genetic integrity of the two species. This pattern of assortative mating could explain the lag time between the introduction of mallards and widespread hybridisation, and why the hybrid population, when reaching a certain threshold, then appeared to increase very quickly. As this observed pattern was predicted by my mathematical model, it seems likely that assortative mating between hybrids may help drive the formation of a hybrid swarm.

The grey duck and mallard hybridisation in New Zealand: current situation and future outlook

The current population of mallards and grey ducks in New Zealand is a hybrid swarm. Grey ducks might well be extinct already in the pure form, and any remnant populations are likely to vanish soon where they survive. The current swarm is more mallard-like than grey-like both in appearance, and in its genetic composition. No large selection pressures on health could be found here, but there is likely to be selection of some type on the swarm at present that is favouring the mallard. The larger clutch sizes of mallards may indicate higher productivity and there also appears to be a mating

advantage for males of a mallard-like appearance. It is therefore possible that the swarm will develop towards a more mallard-like plumage, at least in males, a process that might favour other traits associated with the mallard genome.

In future, there will probably be no pure grey ducks in New Zealand. Instead, there will be a swarm of hybrid individuals that are likely to remain highly variable for a while, and then converge more and more on one type. This type is likely to be mallard-like, but noticeably not pure mallard. It may even possibly resemble the Mariana's mallard (*Anas oustaleti*), an extinct population of ducks that is thought to have arisen from a hybridisation of mallards and grey ducks [Yamashina 1948, del Hoyo et al. 1992]. In this case, New Zealand might be, to some degree, witnessing the recreation of the Marianna's mallard. It will in itself be interesting to see how much those two populations differ.

Should we be concerned about loosing the grey duck in New Zealand, or put differently, is the hybridisation a conservation problem? The relative evolutionary potential of the swarm relative to the parental species is unknown. It is possible that the hybrids combine adaptations of the mallard and the grey duck that together suit them well to the modern environment and condtions of New Zealand. However, the fact that the hybrid swarm appears to develop towards a population overall similar to the mallard suggests that substantial characters of grey ducks are being lost. Since both species used to do well – the grey duck was a common species dealing well in a modern landscape – it is reasonable to suggest that the hybridisation will leave just one population where once there were two quite distinct ones, and this is likely to reflect a loss of evolutionary potential. There is therefore little reason to expect the hybrids to be generally fitter or of higher future potential than the grey duck would have been, although this cannot be said

with certainty. Even given some uncertainty, however, the fact remains that the mallard is an introduced species threatening a native – a situation generally considered undesirable in conservation. Ethically, there is little reason to view the threat to the grey duck by hybridisation with an introduced species any different than if grey ducks were being predated to extinction – the mode might be a different one, but the result is nonetheless the disappearance of a native species.

Moreover, it can be regarded as particularly tragic that one of the few New Zealand native bird species that adapted well to humans, that thrived in agricultural and urban landscapes and was a familiar sight in city parks, is vanishing silently and without much notice being taken or attempts made to rescue it. Even conservation specialists are often not aware that a problem exists [personal observation].

Implications for the grey duck globally

It can also not be assumed that grey duck extinction via hybridisation will be limited to New Zealand. The grey duck is probably threatened as a genetically isolated species by this hybridisation. The population and subspecies in New Zealand are likely to not persist in the wild for another decade, and mallards exist in other areas of the grey duck's range, most notably in Australia [Marchant and Higgins 1990]. A previous conclusion that there was no risk to the grey duck in Australia unless more mallards were introduced [Braithwaite and Miller 1975] relied fundamentally on the assumption that hybrids between the species might suffer reduced fertility and viability [Williams and Roderick 1973]. These data were later re-analysed and the conclusion that grey duck x mallard hybrids suffer lower fertility has been disputed, also calling in doubt the conclusion that

grey ducks are not in danger in Australia [Haddon 1984]. Additionally, the sensitivity analysis conducted here suggests that, to make long-term co-existence likely, values for reproductive output, mate choice, or mortality need to be outside the extremes of those reported in the literature or assumed as reasonable. In my opinion, it is unlikely that the encounter between these two duck species in Australia will end differently in the long run than it did in New Zealand. Conditions, particularly habitat, climate and predation, might differ from those in New Zealand [Braithwaite and Miller 1975], and may even slow the establishment of a hybrid population, but I fear they are unlikely to be so fundamentally different between the two countries as to make it impossible for hybrids to establish themselves.

If the species is to be saved, action needs to be taken. Aside from captive breeding, the only option that appears viable at the current time may be the control of mallards on isolated islands. Occasionally, mallards might re-colonise [Marchant and Higgins 1990], but if this is monitored it should be possible to remove the few immigrating individuals. It is unrealistic, at this point in time, to expect to rescue the New Zealand subspecies on the mainland at the present time. Even if a sufficient number of individuals could be found, there are no areas that mallards and hybrids will not reach, and public pressure and hunting interest would prevent mallard culls.

The grey duck and mallard hybridisation in the context of global waterfowl hybridisation

The Anatidae are one of the most commonly hybridising groups of birds, and probably of all animals [Johnsgard 1960, del Hoyo et al. 1992]. Rates of hybridisation

have probably naturally always been high, and are of evolutionary consequence [Johnsgard 1960]. For example, mitochondrial DNA of falcated ducks (*Anas falcata*) appears to have migrated into gadwalls (*A. strepera*) [Peters et al. 2007]. One interspecific duck hybrid is even of commercial importance, the mule duck or moulard, which is the source of a duck-based *foie gras*, is a sterile cross between mallard (*A. platyrhynchos*) and muscovy duck (*Cairina moschata*) [Delacour 1964, Donkin 1989, del Hoyo et al. 1992].

However, hybridisation in many waterfowl species today has increased or even been induced due to human influence and activities. The mallard, in particular, has spread around the globe by following humans, as it does well in agricultural and urban landscapes, and it has also been introduced deliberately as a game bird. High rates of recent hybridisation with mallards are considered a conservation threat for the Hawaiian duck (*A. wyvilliana*), the American black duck (*A. rubripes*), the mottled duck (*A. fulvigula*), the Mexican duck (*A. diazi*), Meller's duck (*A. melleri*) and the yellow-billed duck (*A. undulate*) as well as the grey duck (*A. superciliosa*) [Browne et al. 1993, Drilling et al. 2002, Rhymer 2006], and hybridisation with other species such as the eastern spotbill (*A. (poecilorhyncha) zonorhyncha*) [Kulikova et al. 2004], might turn out to be problematic in future.

These hybridising species of ducks have sometimes been considered to be subspecies of the mallard, due to the very fact that they hybridise widely with them [Johnsgard 1961], thus reducing the need for conservation by a change of definition. There is considerable controversy and uncertainty concerning the phylogeny of the Anseriformes, quite possibly partly as a result of ancient hybridisation that may have led to transfer of

genes and particular mtDNA [see e.g. Johnson and Sorenson 1991, Donne-Goussé et al. 2002]. However, genetic studies have revealed substantial differentiation within the genus *Anas* [see e.g. Hawaiian duck and mallard; Browne et al. 1993; Meller's duck and mallard; Young and Rhymer 1998], with particularly the African and Pacific clades appearing well differentiated [Johnson and Sorenson 1991]. The grey duck appears significantly distinct from the mallard with mitochondrial DNA sequence divergence being estimated at 7-11% [Rhymer et al. 2004]. The ability of duck species to hybridise is often retained after very long evolutionary time periods, and a consensus is emerging that an ability to hybridise does not negate species status [Rhymer 2006].

As in New Zealand, in each zone of contact with mallards, the other duck species typically suffers from introgression of mallard genes and declines as a pure type, rather than vice versa, and in most cases, mallards were introduced or expanded into the range of other species. Exceptionally, eastern spotbills expanded their range into that of mallards, possibly following human alterations of the landscape [Kulikova et al. 2004]. In consequence, the integrity of some eastern spotbill populations may be threatened by introgression, as again it appears that mainly the non-mallard species is being introgressed [Kulikova et al. 2004].

The North American black duck and mallard hybridisation: a well-studied example

The hybridisation between the North American black duck and mallard has received significant scientific attention. Nonetheless, the extent of hybridisation long remained unclear, highlighting the difficulties of establishing even basic facts when it comes to hybridisation. In Ontario, a dramatic increase in mallard numbers relative to black ducks

was recorded from 1971 to 1985 [Ankney et al. 1987], but no change was found in relative proportions of both species from 1992 – 1993 to 1999 – 2002 in Cape Breton Island in Nova Scotia [McCorquodale and Knapton 2003]. However, both counts were based on plumage characters, and genetic data showed the hybridisation to be so advanced that the two species are no longer genetically recognisable as distinct taxa, while historic museum skins reveal a much more marked genetic distinction [Mank et al. 2004]. It can therefore be assumed that many individuals in the surveys were in fact neither mallards nor black ducks, but hybrids. The American black duck appears to have disappeared into a hybrid swarm in a matter of a few hundred years at most, a fact that underlines the potential threat to the grey duck and other species.

The underlying causes and mechanisms of hybridisation between the American black duck and mallard are not clear despite considerable study. For example, McAuley et al. [1998] did not find a marked advantage for either species in aggressive encounters. One study found an advantage of mallards in reproductive parameters [Maisonneuve et al. 2000], but another did not [Longcore et al. 1998]. Forced copulations were found to be unimportant relative to mixed pairings by one author [d'Eon et al. 1994], but were observed frequently and judged to be important by another [Seymour 1990]. It is possible that this reflects significant regional differences in hybridisation rates, a phenomenon not unknown in hybrid zones [Gerber et al. 2001, Parris 2001], but it is also possible that the studies were conducted at a point in time when the two species were considerably introgressed already, and the different types in their original form had ceased to exist. Though plumage colouration might have been very variable, just as in the grey duck and mallard in New Zealand, this does not have to reflect underlying genetic hybrid classes. It

remains unknown what drives hybrid swarm formation and genetic assimilation in this case, and it might now be too late to find out.

The ruddy duck and white-headed duck hybridisation: an example of attempted management

One case of duck hybridisation which appears to move towards a more hopeful conservation outcome is that of the native white-headed duck (*Oxyura leucocephala*) and the introduced ruddy duck (*O. jamaicensis*) in Spain. Ruddy ducks were introduced to Europe from America as ornamental birds, but have become established in the wild. It had been suggested that they colonised Europe naturally, but molecular studies were able to refute this hypothesis [Muñoz-Fuentes et al. 2006], and eradication programs have been started in many regions. A mathematical model has suggested that sustained culls could reduce the numbers of ruddy duck to below 50 in the UK as quickly as within a few years [Smith and Henderson 2007], and importantly a genetic study found that while hybrids and backcrosses are numerous, there are no signs of major introgression at this point in time, and that the current eradication program indeed seems to prevent or reduce hybridisation [Muñoz-Fuentes et al. 2007]. This case shows that with vigilance, and quick and determined measures, it is possible to recognise and manage unwanted hybridisation in the wild, which might give hope for other species.

Lessons for conservation

A main difference between the case of the white-headed duck and those quoted before is that the white-headed duck was considered endangered even without

hybridisation, while most of the other species are considered numerous and of least concern up to the present day (with the exception of the Hawaiian duck). Ironically, being endangered proved an advantage for the white-headed duck, as a further reduction in numbers by hybridisation was quickly recognised as a threat and conservation agencies acted fast. Sadly, the criteria used by the IUCN to declare a species vulnerable or endangered which are based on reduction of individual numbers below certain thresholds, or decline exceeding a certain speed, may not be adequate when dealing with the threat of hybridisation. For example, in a hybrid population it might be impossible to estimate the numbers of each 'pure' species, and these numbers are likely to be overestimated in many instances. The case of the American black duck discussed above shows that in the absence of genetic data, it is possible even for scientists studying hybridisation to misjudge the seriousness of the situation. More critically, however, once hybridisation has reached a certain threshold, it might be too late for any effective measures, or the effort needed might be prohibitive. For example, even if it were accepted now that the numbers of grey ducks have declined to a dangerous level, the trend is likely to be irreversible as it will be nearly impossible to remove hybrids and mallards from areas of sympatry. To consider this species as to be of least concern does not reflect the global threat it experiences from hybridisation, which, if unchecked, might exterminate it in its pure form within a century or so. Contrary to a threat from habitat destruction or overharvesting, the point of no return for a threat from hybridisation can be reached while there are still quite high numbers of seemingly pure individuals present.

Hybridisation in conservation: too much, too little, or just right?

Interest in hybridisation has increased dramatically in last decade [Schwenk et al. 2008], leading to the identification of and increased attention to a range of challenges that hybridisation poses to conservation. As the scale of the problem is being appreciated, the need for research is becoming more apparent, with the ultimate aim to establish firmer guidelines for best practise on how to deal with these challenges, and with concrete techniques for their implementation.

Hybridisation offers two directly opposing conservation problems: (1) those caused by unnaturally high levels of hybridisation that can threaten the integrity of species or subspecies, and (2) that of unnaturally low levels of introgression when human activities curtail the opportunities for hybrid pairings, thus restricting natural gene flow. It is important to realise that it is not correct to assume that hybridisation will always be detrimental to conservation, while in some cases it is clearly so. It is not surprising that this situation can cause confusion within conservation agencies and their personnel, as well as in the wider public. The idea that naturally occurring gene flow is a feature worthwhile of conservation in itself has only been established relatively recently, with articles in high profile journals attempting to raise the profile of the issue, both for plants [Whitham et al. 1991] and animals [deMarais et al. 1992].

While decreased rates of gene flow may slow or alter ongoing evolutionary processes, the more imminent, more dramatic danger, stems from increased rates of hybridisation. Large scale increased hybridisation can reverse evolutionary processes of hundreds of thousands or even millions of years in an evolutionary eye blink of few generations. Incipient species that developed separate adaptations over significant amounts of time

can rejoin, or species might simply disappear and leave no significant, genetic heritage behind. While in cases of decreased hybridisation, genetic contact between populations might increase again in the future, or be increased by human influence as a conservation measure, the consequences of cases of increased hybridisation can quickly turn irreversible. In fact, widespread hybridisation caused by humans might be one of the most difficult conservation problems to manage.

The first hurdle: recognising hybridisation

Even to identify if there is a problem or not in the first place can be difficult. Hybridisation can go unnoticed, as the appearance of hybrids is often less obvious to the public than the absence or reduction of a species. Despite ducks being one of the most urban of bird species, and arguably one of the most familiar to the average person, in my experience a surprisingly low number of people have noticed the dramatic change in the appearance of New Zealand ducks during their life times. In most other cases of hybridisation, the two species will possess phenotypes that are a lot less diverged, and be much less familiar to people, and thus, hybrids will be even less likely to be noticed. If abnormal variants, that might be hybrids, are found and brought to the attention of the relevant agencies or individuals, it still remains a problem to clarify if they really are hybrids.

My study shows once more the necessity of not relying on phenotype alone when identifying and classifying hybrids. Cryptic hybrids will be missed with a phenotypic approach, colour variants of the pure species could be falsely declared to be hybrids, and phenotypic scores are often unreliable as estimator of intermediate hybrid classes [see

e.g. Bensch et al. 2002, Pfenninger et al. 2002, Randi and Lucchini 2002, Bronson et al. 2003, Thulin et al. 2006]. In most cases, genetic identification is probably the only safe identification. Unfortunately, genetic identification requires time, knowledge, resources, and is comparatively expensive. If a hybrid problem is suspected from phenotypic observations, it is probably still desirable to seek molecular confirmation. In the case of grey duck and mallard in New Zealand, widespread hybridisation was well documented before this study and it has become clear that it is ongoing and will continue until all pure grey ducks disappear.

The second hurdle: determining if action is needed and sensible

Once increased hybridisation has been confirmed, it is an awkward conservation problem to deal with. Action can be very time critical. The larger the hybrid proportion in the population is allowed to grow before measures are taken, the less likely it is that it can be stopped or reversed. Just a few breeding seasons can make the difference between a localised, relatively easy to contain development, and a population-wide catastrophic one. At this stage, modelling might be a useful tool to predict population development and to focus further research and evaluate management options. Unfortunately, in many cases insufficient details of the populations will be known to allow adequate modelling. To gather the data will be feasible only in few high profile species, as it was for the case of red wolves (*Canis (lupus) rufus*) and coyotes (*C. latrans*) [Fredrickson and Hedrick 2006].

In general, a conservation manager will have little data to help him, or her, determine the best strategy, but a large number of difficult questions to answer. First of all, is there a

need to intervene in order to prevent unwanted outcomes? In some cases, limited hybridisation might occur but will never develop into a problem. Hybrids might, for example, be sterile or sexually uncompetitive, in which case it is not usually advisable to use scarce resources in order to find these individuals. Genetic studies might help identify backcrosses [see e.g. Mueños-Fuentes 2006], which would be a clear indication that introgression might become a problem.

For the grey duck, introgression does occur on a scale wide enough to be problematic. In fact, it is doubtful that any viable populations of grey ducks survive. Observations as well as the modelling approach furthermore suggest that if the species is to be rescued, action will have to be taken.

The third hurdle: what action is to be taken?

If intervention is warranted, what should be done? Should there be destruction of hybrids? If so, how are hybrids to be identified, considering the unreliability of phenotypic criteria, which are even exacerbated in the field where animals are often difficult to approach? Or alternatively, might it be easier to identify the underlying cause for increased hybridisation, and possibly remove it? If a previously allopatric species has been introduced, can it be eradicated? If two species have been brought in closer contact by habitat alterations, can environmental measures be successful in restoring the barriers? If the numbers of one species had dropped dramatically in the area so they cannot find partners, might it help to increase their numbers by introductions, habitat measures, or even feeding? Finally, is there much hope of success, or is the situation too far gone and are attempts likely to be a waste of resources? In some cases, captive breeding might be

the only viable strategy, at least as an emergency measure. In the end, each case is likely to differ from the next, making guidelines complex and generalised advice difficult [Allendorf et al. 2001].

There is no question that the more is known about the species involved and the causes of hybridisation, the more informed those decisions are likely to be. An example in this study highlighting the importance of detailed knowledge is the unexpected tendency of hybrids to mate assortatively with other hybrids. This fact suggests that in this case a build-up of a hybrid population is more likely than the more usual introgression via backcrossing. Therefore, a quick increase in hybrids is to be expected as a threshold is reached. This threshold will be that number of hybrids that enables most hybrids to find other hybrids as partners. If this had been known earlier, a low proportion of hybrids in the population might have been taken more seriously as a threat, and it should be seen as such from now on in areas of co-occurrence of these two species. Knowledge of hybrid mate choice patterns, so rarely studied to date, should be given more attention as it might enable more accurate prediction of which cases of hybridisation will result in limited introgression, and which may endanger the integrity of species through the formation of hybrid swarms. Information regarding many other determinants might be of just as much interest, however, critical determinants are likely to vary between cases.

Genetic restoration

Eventually, the question of genetic restoration might be raised. In some widely hybridising populations it might be possible to collect some pure individuals for captive

breeding, or for release in areas where hybrids are absent or can be controlled. Again, the problem of identification of pure individuals is nontrivial. The question is also if there is any long-term perspective for a conservation effort. Are there areas where wild populations can be established without a forbidding risk of renewed hybridisation? If not, a captive breeding program would be of limited value. This might be the case for grey ducks in New Zealand. At the current point in time, plans to re-establish the species on the mainland are probably utopian. Mallards and hybrids are too widespread, and too mobile to allow establishment of reserves on the mainland. It is unlikely that there would be public support for any culls, particularly as hunters prefer mallards to the smaller grey ducks as game, and considerable populations exist in urban parks. However, smaller offshore islands far from large landmasses might offer a chance for the establishment of grey ducks in the wild. Mallards are capable of long distance dispersal over sea, and have colonised most islands on which grey ducks occur [Marchant and Higgins 1990], but occasional colonisers could be monitored and controlled.

In fact, a study has already assessed the feasibility of eradicating the entire duck population, all of them hybrids, of Lord Howe Island, and recommended that this project is to be implemented [Tracey et al. 2008]. It is unclear, however, what is planned after this eradication. Natural re-colonisation of grey ducks from Australia might be possible, but the arrival of mallards or hybrids is just as, if not more, likely. Continued monitoring will therefore be required. Alternatively, grey ducks could be introduced. Phenotypically pure individuals from Australia or some Pacific islands could be collected, and then be tested using molecular methods like those employed in this study to verify that they have a high chance of being pure grey ducks. Lord Howe Island might thus be turned into a

sanctuary for the grey duck, befitting the status of the Lord Howe Island Group as a UNESCO world heritage site.

The conservation value of an introgressed population

Increasingly, there is also the question if low levels of introgression might in some cases be acceptable for recovery of a species. In the case of Leon Springs pupfish (*Cyprinodon variegatus*), for example, all four wild populations now show signs of limited introgression with sheepshead minnows (*C. bovinum*), and only a small captive population remains pure [Echelle and Echelle 1997]. Would it be acceptable to remove as much genetic material of sheepshead minnows from the natural populations as possible, leading to populations with maybe less than 1% of introgressed material, or is it preferable to exterminate the wild populations and repopulate the areas with descendants of the captive one? Maintenance of the pure species might lead to a quite dramatic loss of its genetic diversity. Is it preferable to maintain a narrow but pure gene pool, or a wider but partly introgressed one? This question has also been raised for some of the North American trout species (*Oncorhyncus apache* and *O. gilae*) threatened by congeners (*O. clarki* and *O. mykiss*) introduced for sport fishing. It has been suggested that exterminating all hybrids would, in some populations at least, remove too large a fraction of the original diversity [Dowling and Childs 1992]. This dilemma is likely to occur in each case where hybridisation is widespread enough to endanger the integrity of whole species or subspecies, and it should be addressed carefully in each case. There might be an immediate desire to exterminate all hybrids and restore the pure species, but in some cases this might turn out detrimental in the long run.

Positive aspects of hybridisation and introgression

It is even possible that sometimes, endangered remnant populations will profit from limited introgression. As shown here using *Drosophila*, hybridisation can increase in inbred populations, which suggests the possibility that it might counteract inbreeding depression, introduce vital adaptations, or increase levels of heterozygosity. More study is needed for firm recommendations, but in the meantime it may be prudent to monitor inbred populations particularly closely for signs of hybridisation, and in populations where hybridisation is a problem, broadening the gene pool by introduction of conspecifics might reduce hybridisation rates. However, it also leads to the question if limited hybridisation is not desirable in inbred populations when no conspecifics are available. For example, if the population is very small, it might aid recovery from inbreeding and a limited gene pool. Indeed, it appears that hybrids of endangered Forbes' parakeet (*Cyanoramphus forbesi*) with red-crowned parakeet (*C. novaezelandiae*) have improved immune function over the relatively inbred Forbes' parakeet, and it has been suggested that low levels of introgression might profit the health and resilience of this species, while large levels are still undesirable [Tompkins et al. 2006]. Since limited introgression is known to be a natural process in many populations, and might be an adequate coping mechanism in times of crisis, perhaps hybridisation should not always be seen as altogether negative or as diminishing the conservation value of a population.

Conserving populations with a high level of hybridisation

Sometimes only hybrids might be available for restoration. Is such a population worthless in terms of evolution and conservation, or does it retain a value as it does carry

unique genetic material, if only in admixture? This might be a philosophical question rather than a scientific one (as are a number of questions raised in the preceding and following paragraphs), but it needs addressing as it has far reaching consequences. To some degree the answer probably depends on the level of introgression, and the amounts of genetic material of the original species salvageable. It might also depend on how different the species involved are from each other. Another consideration might be how unique the threatened species is, and even how iconic it is.

For example, most people would agree that the Przewalski horse (*Equus ferus przewalskii*), the last extant species of wild horse, is worthwhile rescuing although one of the 12 founders of all current individuals was a hybrid with a domestic mare [Groves 1994, IUCN Equid Specialist Group 1996]. Attempts have been made to eliminate phenotypic signs of this ancestor from the breeding program [Groves 1994], but undoubtedly the genetic contribution cannot be removed. Although there is very little genetic material of the original Przewalski horse left, wild horses are a very iconic species that people feel an emotional attachment to, and they also represent a rare remnant of the Eurasian megafauna.

While the grey duck might be rescued on islands, or in captive breeding programs, what is to happen with the current hybrid swarm on the mainland? There is probably not much of a choice – it is unlikely at this point in time that any process can reverse the level of hybridisation or restore the grey duck. However, does this hybrid swarm have a conservation value? In my personal opinion, and this is a question that can only be answered by each person individually, the value of the mixed swarm from a species conservation view (for an ecological view, see below) is very limited. The overall

character of the swarm is mallard-like, and very few grey duck features are preserved. If no grey duck can be rescued anywhere, the value of the swarm would climb somewhat, but I expect that once a genetic equilibrium is reached, there will be too little left of the grey duck to be worth conserving. Any money and effort are better invested in other species or, if available, other populations of the same species.

The importance of the ecological role of a hybridised population

Possibly the biggest issue that needs addressing in the management of hybrids, however, is the ecological role of the species in question, and how much of that role will be preserved. A hybridised population might be able to fulfil its ecological role just as well as a pure one, while its removal or reduction might have dire consequences. 'Purity' of a species is a human concept rather than a biological one. If hybridisation leads to a shift in ecological niche, this merits particular attention. It might lead to invasiveness [see e. g. Lewontin and Birch 1966, Durand et al. 2002, Facon et al. 2005], and to follow-on effects on predators, prey, and any other member of the same ecosystem. These consequences might be quite unpredictable, as much as are those of the removal of a species. Unfortunately, little is known about such ecological effects of hybridisation, as they have hitherto not received much scientific attention. Such consequences might arise from the hybridisation of grey ducks and mallards. Mallards are substantially bigger than grey ducks, and so are the hybrids. This increases the water depth to which they can forage by upending [Haddon 1998], possibly by as much as 10 cm. For invertebrates, small vertebrates and plants living in the shallow water zone, this could be quite a dramatic change. Predation pressure from ducks on some species might increase, and the

vegetation might be altered substantially at that depth. It is entirely unknown what the effect is likely to be, but ducks being as common as they are, it is possible that it will not be negligible.

When it comes to assessing a hybrid or introgressed population's ecological role, it might also be important to consider the changes those habitats have experienced from humans. In some cases, it is a theoretical possibility that hybrids increase as a response to human habitat alterations that favour new, highly adaptable genotypes. For example, the modern New Zealand is very different from the country prior to the arrival of humans about 1000 years ago, and even from the arrival of Europeans about 300 years ago. There are now mammalian predators, many native species have become extinct, many non-native animals and plants have invaded the entire country, forest cover has decreased dramatically, and agricultural areas and human settlements are now the defining features of the landscape in large areas [Halkett 1991, Garden 2005]. To a certain degree, the current habitat is a mosaic of features of pre-settlement New Zealand on the one hand and European and North America elements on the other. Possibly hybrids do well because in an intermediate environment they combine adaptations for different parts of the mosaic environment. Even though it does not appear to be the case that grey ducks prefer undisturbed habitat to agricultural land, it is still possible that certain combinations of adaptations are advantageous in the hybrid swarm.

Hybridisation as an adaptive evolutionary response

If it were the case that hybrids do well on intermediate habitat or habitat disturbed by human activities, in this species pair or others, hybridisation might be an evolutionary

adequate response to the rapidly changing environment. It makes sense to expect these often dramatic changes to induce strong selection pressures and change the course of evolution by favouring very different adaptations from those previously advantageous. If hybridisation can speed up such evolution, then for some species, selection might favour hybridisation as a way to rapidly acquire diversity and genes to cope with a rapid rate of change. This further complicates the question of when hybridisation is a desirable or undesirable feature for conservation. Species are not immutable, and it would be presumptuous of conservationists to try to preserve them as static entities. If humans alter a species' habitat, they will alter its fate in the long term. Should we then attempt to preserve the species as it was, in an environment it is no longer adapted to, or are there cases in which the rapid alteration of a species should be seen as a natural process that increases a population's fitness, and therefore, its long term chances of survival? If so, how do we distinguish between these and other cases? There is probably no right or wrong view in this dilemma. In my personal opinion, if the two hybridising species would not normally have had the chance of contact, the hybridisation should be viewed as unnatural and undesirable, but in other cases it might well be that the prevention of change is just another intervention of humanity into a natural system that is adapting to new environments. In the end, the decision will have to be taken individually in each scenario, but all options should be considered.

The consequences for global biodiversity

So what are the likely global consequences of human caused changes in hybridisation rates? While locally, it might increase or decrease diversity [Seehausen et al. 2008],

globally a net loss of genetic diversity is likely. But even more, as hybridisation rates increase in many parts of the world, and as incipient species merge, it is not only current genetic diversity that is lost, there is also a likely net loss of evolutionary potential, and therefore of future species [Myers and Knoll 2001, Rosenzweig 2001]. Homogenisation of gene pools reduces local adaptations, and the number of subspecies. It therefore reduces the potential of species to split into reproductively and ecologically separated units in the future. Therefore, the increased rates of hybridisation today, together with the rapid rate of extinction of species, subspecies and populations, is likely to influence evolution for a long time even after these processes themselves have stopped. There will simply be less diversity, and fewer distinctive populations, and therefore fewer separate starting points from which new forms can arise. The loss of evolutionary potential is a serious further influence of humanity on the environment, the extent of which will show in the far future, and might be impossible to measure.

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Appendix 1

Plumage score used to categorize mallards, grey ducks and their hybrids

[from: Rhymer JM, Williams MJ, Braun MJ. 1994. Auk 111(4): 970-978. Mitochondrial Analysis of gene flow between New Zealand mallards (*Anas platyrhynchos*) and grey Ducks (*Anas superciliosa*)]

Points for all five areas are determined for each individual and added together for the final score (maximum 25 for females, 26 for males)

Face

0 – two black stripes, the upper superciliary stripe of uniform width and extending well beyond eye; lower stripe tapering from gape to below eye and giving way to mottled cheek; clean cream stripe between eye and crown, throat and face cream

1 – two black stripes, upper stripe as described above, lower stripe merging with mottled face midway between bill and eye, clean cream stripe between eye and crown; face and cheek mottled cream but with obvious cream patch at base of bill between the two black stripes

2 – superciliary stripe obvious and extending from bill to well beyond eye, lower stripe absent but small patch of black feathers at bill base at gape; mottled cream stripe between eye and crown, face and cheek entirely mottled black on cream background, throat cream

3 – entire face mottled black on fawn ground, superciliary stripe indistinct and narrow small patch at gape more heavily mottled to appear darker than rest of face, small fawn patch at bill base between gape and bill top, throat clean to lightly mottled fawn

4 – Dark mottled face and throat, black on fawn background in female, blackish-green on dark fawn in male; no obvious eye stripe, no fawn patch at bill base

5 – Predominantly dark green head, face and throat (males only)

Legs

1 – dark olive greenish-brown
2 – khaki
3 – yellow-orange to dull orange
4 – bright orange
5 – red orange

Bill

0 – uniformly black
1 – black with very dark green particularly at base and along edge of upper mandible
2 – predominantly black/dark green, some yellow or brown at tip
3 – black and brown/yellow
4 – yellow green
5 – green or a bluish shade (common in NZ mallards)

Speculum

0 – green, no discernible bar
1 – green, obvious thin and narrow whitish/brown line
2 – green, bar distinct but narrow and mottled fawn colour
3 – purple, bar distinct but narrow and mottled fawn colour
4 – purple, bar distinct, wide, mottled fawn
5 – purple, bar narrow, pure white
6 – purple, bar wide, pure white

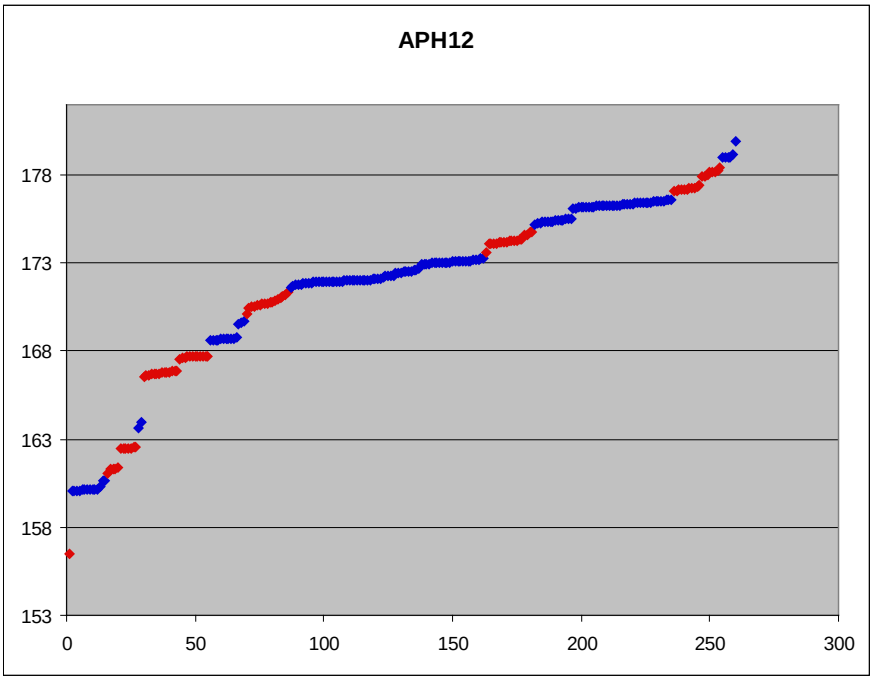
Posterior border to speculum

1 – black
2 – black followed by thin (1mm) white line
3 – black followed by narrow (1-2mm) white line
4 – black followed by conspicuous (3-4 mm) white bar
5 – black followed by wide (>4mm) white bar

Appendix 2

Flexibin allele binning graphs and tables

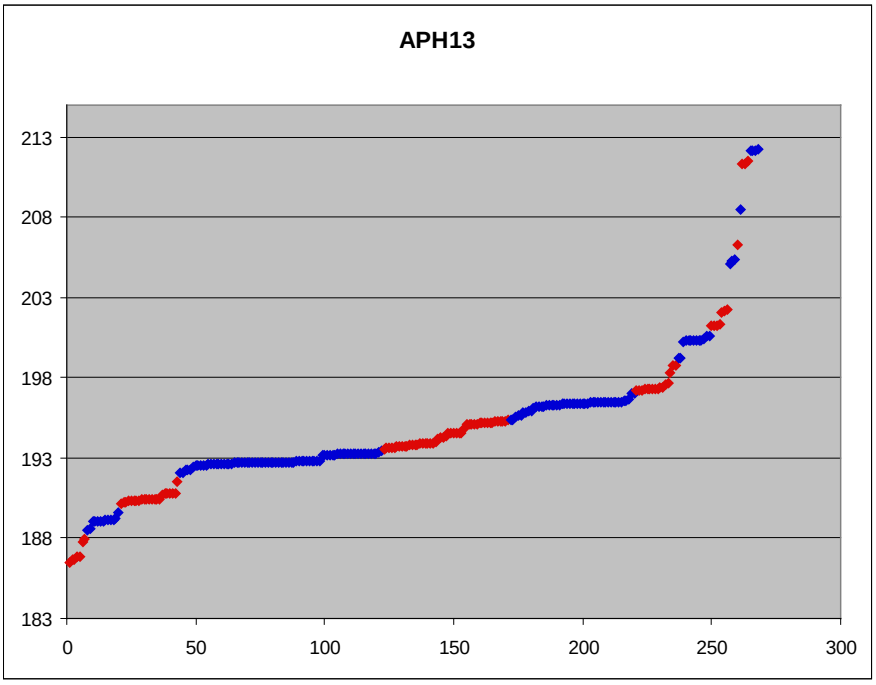
APH 12



	Adjust	Estimated repeat length		
	1,215	1,77499998		
Repeats	Length	Mean bp	s.d.	Count
1	156,286239	156,506239	1	
3	159,886239	160,21	0,205	14
4	161,686239	161,98	0,632	12
5	163,486239	163,79	0,217	2
7	167,086239	167,17	0,481	26
8	168,886239	168,87	0,394	14
9	170,686239	170,77	0,302	17
10	172,486239	172,43	0,513	76
11	174,286239	174,27	0,260	19
12	176,086239	176,07	0,456	54
13	177,886239	177,61	0,474	19
14	179,686239	179,19	0,368	6

No alterations were performed

APH13

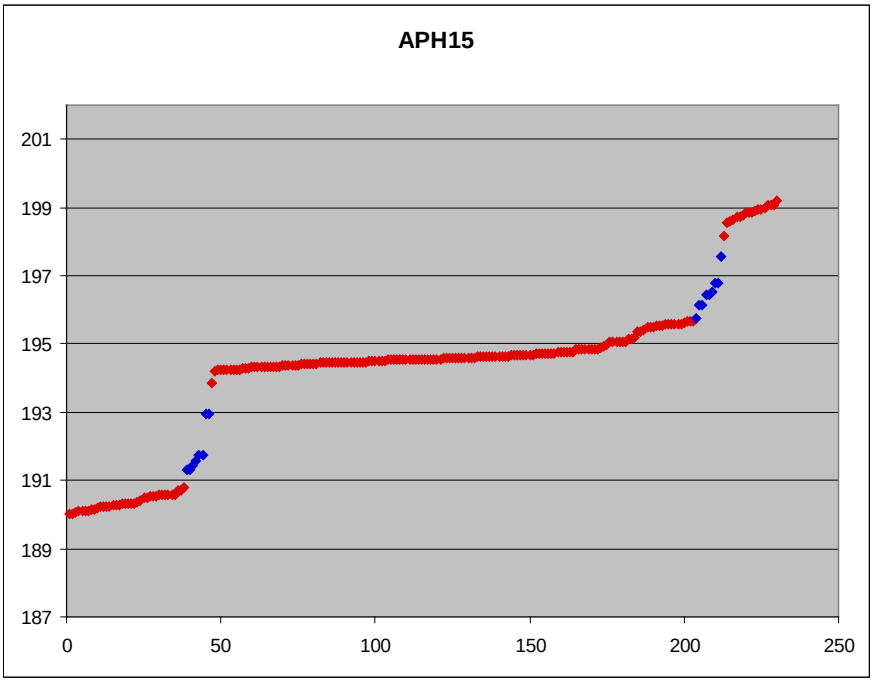


Adjust	Estimated repeat length
0,17	1,80499995

Repeats	Length	Mean bp	s.d.	Count
2	187,262094	187,01	0,590	7
3	189,062094	189,06	0,269	13
4	190,862094	190,51	0,296	23
5	192,662094	192,84	0,317	79
6	194,462094	194,43	0,639	49
7	196,262094	196,30	0,341	49
8	198,062094	197,59	0,519	16
9	199,862094	200,18	0,455	13
10	201,662094	201,66	0,489	7
12	205,262094	205,26	0,111	3
13	207,062094	206,283662	1	
14	208,862094	208,451957	1	
15	210,662094	211,38	0,155	3
16	212,462094	212,18	0,048	4

No alterations were performed

APH15

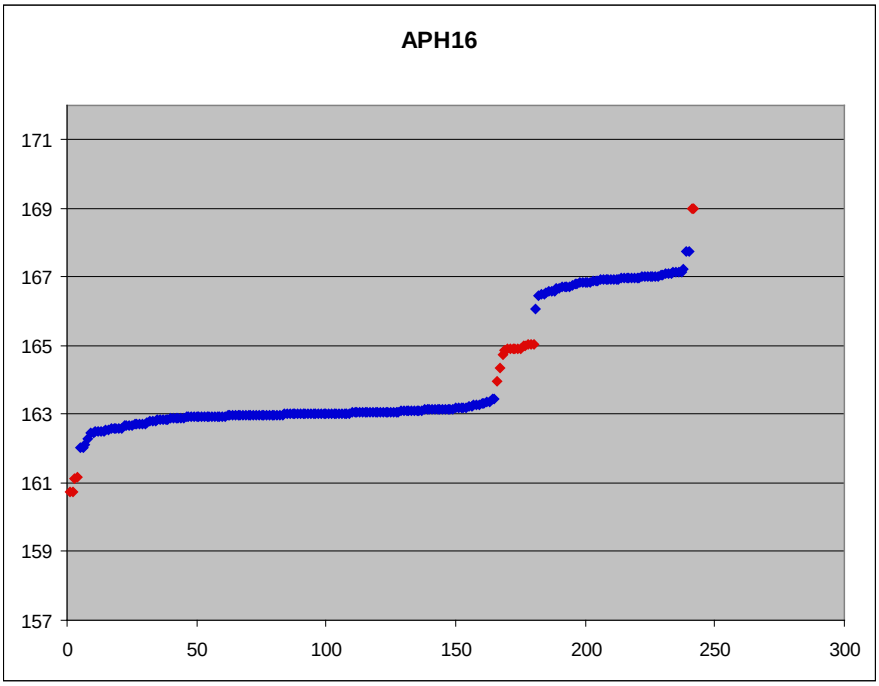


Adjust	Estimated repeat length
1,115	2,17499995

Repeats	Length	Mean bp	s.d.	Count
1	190,207322	190,36	0,213	38
2	192,407322	191,88	0,679	8
3	194,607322	194,69	0,382	157
4	196,807322	196,51	0,520	9
5	199,007322	198,82	0,240	18

Alterations: The allele was grouped into 3 alleles only, with 1 repeat below 192, 3 repeats under 197, and everything above that as 5 repeats.

APH16

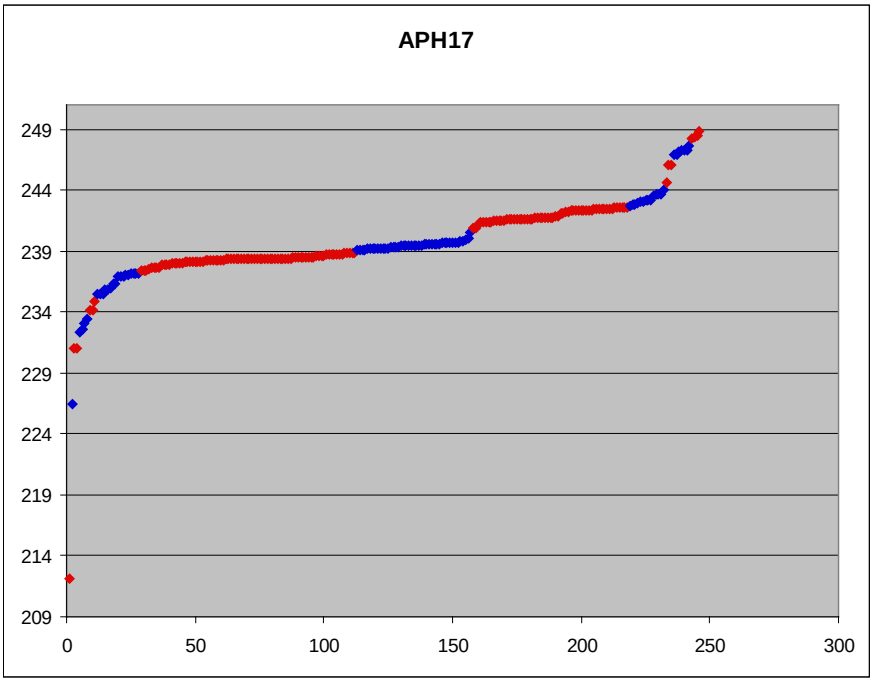


Adjust	Estimated repeat length
1,07	2,05500007

Repeats	Length	Mean bp	s.d.	Count
1	160,784638	160,93	0,235	4
2	162,864638	162,95	0,235	161
3	164,944638	164,83	0,296	15
4	167,024638	166,91	0,263	60
5	169,104638	169,01	0,000	2

Alterations: The highest data point of 5 repeats was included into 4 repeats, and the lowest ones of 1 repeat were included in 2 repeats.

APH17

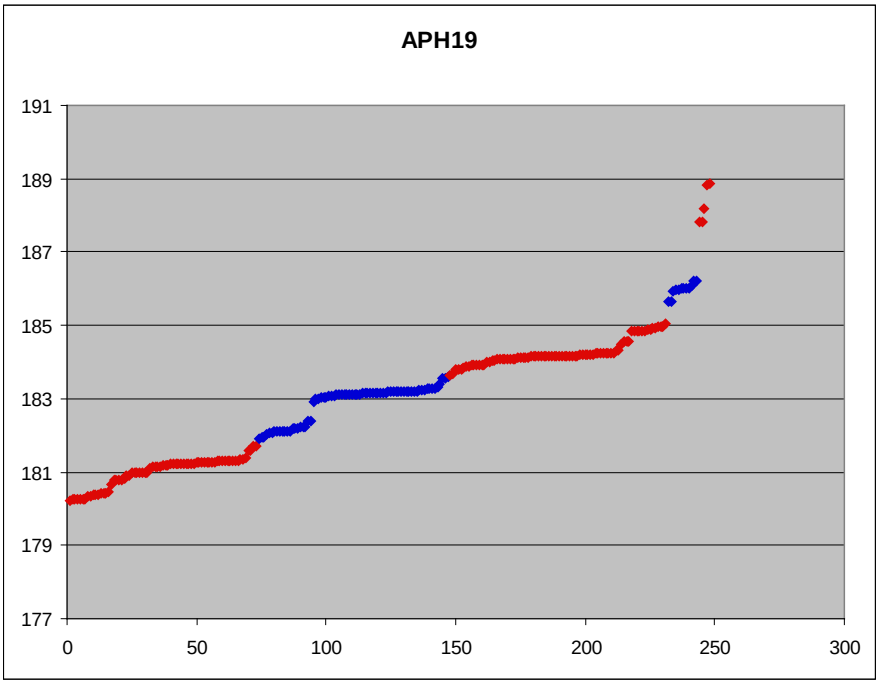


Adjust	Estimated repeat length
0,405	1,78900003

Repeats	Length	Mean bp	s.d.	Count
2	212,934579	212,174579	1	
10	227,334579	226,484375	1	
12	230,934579	231,03	0,000	2
13	232,734579	232,85	0,485	4
14	234,534579	234,40	0,442	3
15	236,334579	236,47	0,675	17
16	238,134579	238,29	0,343	84
17	239,934579	239,48	0,283	45
18	241,734579	241,93	0,466	61
19	243,534579	243,24	0,401	14
20	245,334579	245,58	0,871	3
21	247,134579	247,21	0,250	7
22	248,934579	248,47	0,235	4

Alterations: 14 repeats below 235 were sorted into 13, those above into 15. Also, Repeat 20 below 245 were included in 19, those above in higher 21.

APH19

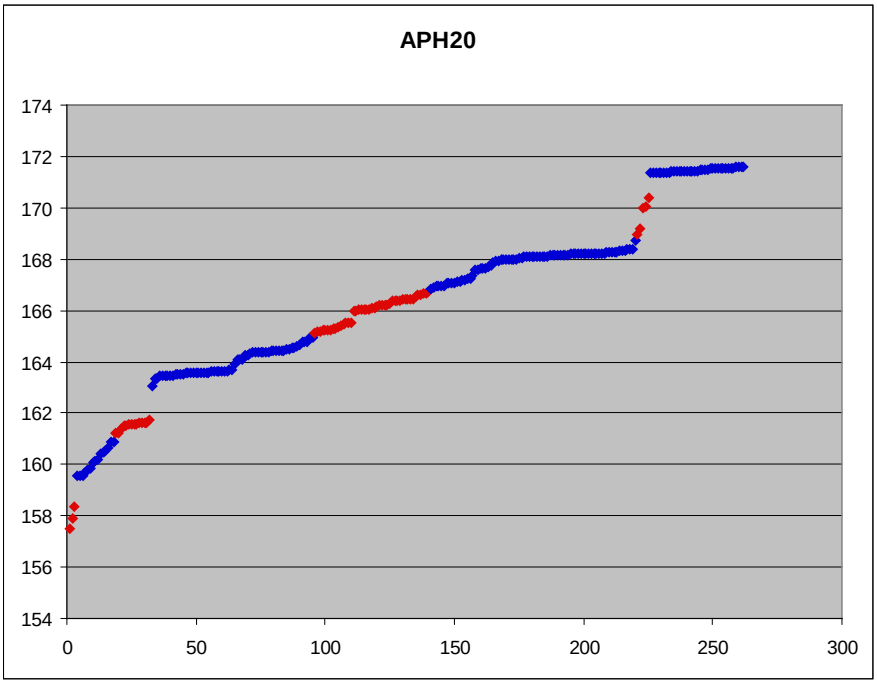


Adjust	Estimated repeat length
0,13	1,77499998

Repeats	Length	Mean bp	s.d.	Count
2	180,885989	180,99	0,402	73
3	182,685989	182,88	0,492	74
4	184,485989	184,25	0,335	84
5	186,285989	185,98	0,179	12
6	188,085989	188,32	0,510	5

Alterations: The three last datapoints of 3 repeats at 183.5 were included in the allele 4 repeats.

APH20

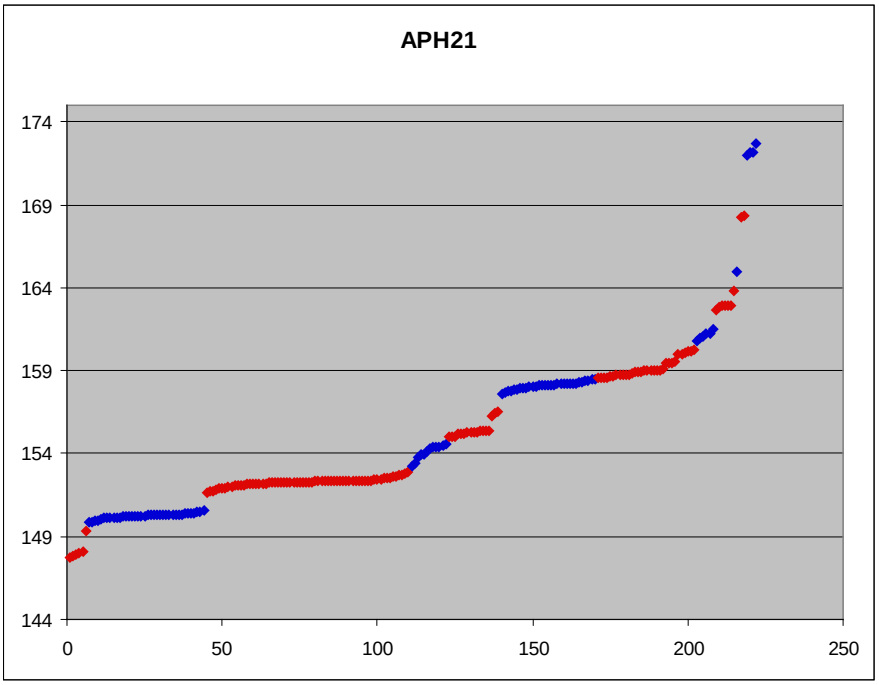


Adjust	Estimated repeat length
-0,155	1,92299998

Repeats	Length	Mean bp	s.d.	Count
2	158,271264	157,89	0,438	3
3	160,171264	160,15	0,474	15
4	162,071264	161,52	0,142	14
5	163,971264	163,98	0,496	63
6	165,871264	165,97	0,511	45
7	167,771264	167,88	0,469	80
8	169,671264	169,70	0,599	5
9	171,571264	171,47	0,077	37

No alterations were performed

APH21

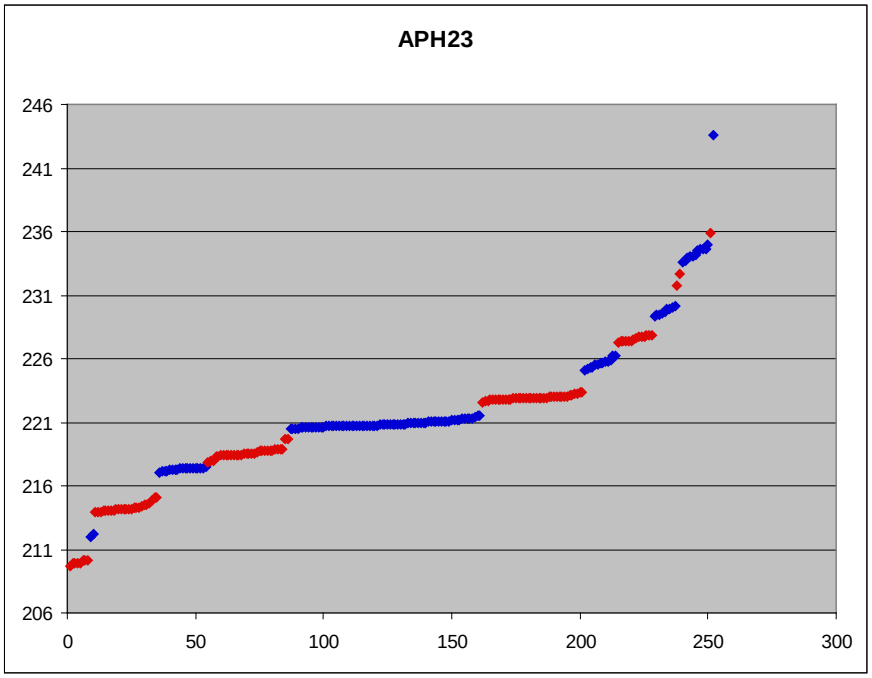


Adjust	Estimated repeat length
0,195	1,81700003

Repeats	Length	Mean bp	s.d.	Count
2	148,523412	148,16	0,601	6
3	150,343412	150,24	0,166	38
4	152,163412	152,27	0,228	66
5	153,983412	154,08	0,414	12
6	155,803412	155,44	0,473	17
7	157,623412	158,09	0,231	31
8	159,443412	159,14	0,536	32
9	161,263412	161,12	0,245	6
10	163,083412	163,00	0,375	7
11	164,903412	164,935065	1	
13	168,543412	168,32	0,083	2
15	172,183412	172,24	0,279	4

No alterations were performed

APH23

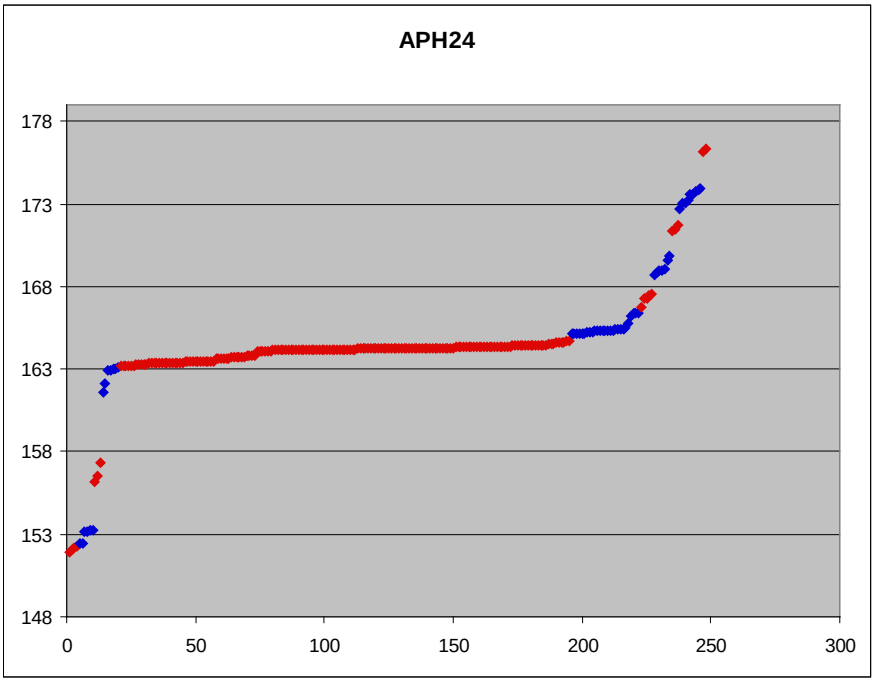


Adjust	Estimated repeat length
1,11	2,19300008

Repeats	Length	Mean bp	s.d.	Count
1	209,852387	209,96	0,160	8
2	212,052387	212,13	0,190	2
3	214,252387	214,28	0,322	25
4	216,452387	217,32	0,113	19
5	218,652387	218,59	0,379	32
6	220,852387	220,86	0,244	75
7	223,052387	222,93	0,174	40
8	225,252387	225,63	0,365	13
9	227,452387	227,57	0,218	14
10	229,652387	229,71	0,280	9
11	231,852387	232,16	0,666	2
12	234,052387	234,25	0,452	11
13	236,252387	235,92645	1	
16	242,852387	243,532338	1	

No alterations were performed

APH24



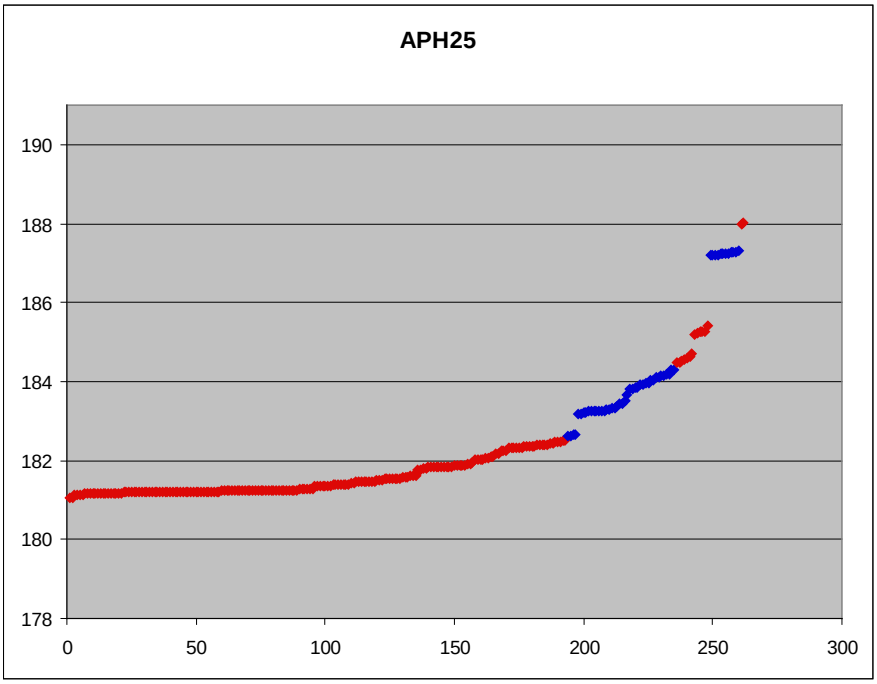
Adjust	Estimated repeat length
0,875	1,77499998

Repeats	Length	Mean bp	s.d.	Count
1	151,41458	152,12	0,160	4
2	153,21458	152,95	0,376	6
4	156,81458	156,65	0,607	3
7	162,21458	162,68	0,587	7
8	164,01458	164,04	0,406	175
9	165,81458	165,46	0,397	27
10	167,61458	167,26	0,293	5
11	169,41458	169,13	0,413	7
12	171,21458	171,52	0,197	3
13	173,01458	173,42	0,423	9
15	176,61458	176,26	0,156	2

No alterations were performed

300

APH25



Adjust	Estimated repeat length
-0,25	1,77499998

Repeats	Length	Mean bp	s.d.	Count
2	181,637037	181,53	0,423	193
3	183,437037	183,55	0,477	42
4	185,237037	184,89	0,370	13
5	187,037037	187,24	0,040	12
6	188,837037	187,99	0,027	2

No alterations were performed

Appendix 3

Supplementary data for chapter 4 and chapter 5

Table 1. Details of all New Zealand ducks sampled for molecular work. If sex was unknown field is marked ?, if sex was uncertain but probably that shown, a (?) follows the assumed sex. Hybrid probability as estimated by Structure.

Sampling season	Dead/Live	Sampling location	Sex	Phenotypic score	Hybrid probability
2006	live	HariHari	?	19	0.879
2006	live	HariHari	female	18	0.835
2006	live	HariHari	female (?)	13	0.904
2006	live	HariHari	male	21	0.887
2006	live	HariHari	female	24	0.767
2006	live	HariHari	?	19	0.808
2006	live	HariHari	?	16	0.865
2006	live	HariHari	male (?)	17	0.894
2006	live	HariHari	?	13	0.813
2006	dead	HariHari	male	21	0.854
2006	dead	HariHari	?	16	0.917
2006	dead	HariHari	?	10	0.356
2006	dead	HariHari	?	17	0.840
2006	dead	HariHari	?	6	0.171
2006	dead	HariHari	male	23	0.854
2006	dead	HariHari	?	19	0.873
2006	dead	HariHari	female	12	0.718
2006	dead	HariHari	female	8	0.092
2006	dead	HariHari	male	16	0.391
2006	dead	HariHari	male	21	0.876
2006	dead	HariHari	male	19	0.329
2007	live	HariHari	female	17	0.830
2007	live	HariHari	male	19	0.833
2007	live	HariHari	male	17	0.930
2007	live	HariHari	male	16	0.957
2007	live	HariHari	male (?)	15	0.893
2007	live	HariHari	?	10	0.519
2007	live	HariHari	male	17	0.855
2007	live	HariHari	?	19	0.789
2007	live	HariHari	male	15	0.655
2007	live	HariHari	?	12	0.371
2007	live	HariHari	?	3	0.164
2007	live	HariHari	?	18	0.626
2007	live	HariHari	female (?)	21	0.652
2007	live	HariHari	?	4	0.159
2007	live	HariHari	male	20	0.780
2007	live	HariHari	?	11	0.669
2007	live	HariHari	?	10	0.566
2007	live	HariHari	?	5	0.480
2007	live	HariHari	male	16	0.844
2007	live	HariHari	male	19	0.796

2007	live	HariHari	male	23	0.896
2007	live	HariHari	male	21	0.304
2007	live	HariHari	male	20	0.946
2007	live	HariHari	?	8	0.135
2007	live	HariHari	?	9	0.445
2007	dead	HariHari	female	9	0.499
2007	dead	HariHari	male	18	0.890
2007	dead	HariHari	female	7	0.094
2007	dead	HariHari	female	19	0.449
2007	dead	HariHari	male	19	0.871
2007	dead	HariHari	female	8	0.209
2007	dead	HariHari	male	11	0.360
2007	dead	HariHari	female	18	0.851
2007	dead	HariHari	male	23	0.617
2007	dead	HariHari	female	8	0.223
2007	dead	HariHari	female	7	0.226
2007	dead	HariHari	female	8	0.434
2007	dead	HariHari	female	16	0.793
2007	dead	HariHari	male	8	0.198
2007	dead	HariHari	male	10	0.356
2007	dead	HariHari	female	18	0.900
2007	dead	HariHari	male	9	0.137
2007	dead	HariHari	female	11	0.354
2007	dead	HariHari	male	14	0.575
2007	dead	HariHari	male	10	0.193
2007	dead	HariHari	female	17	0.514
2007	dead	HariHari	male	25	0.535
2007	dead	HariHari	male	11	0.467
2007	dead	HariHari	male	11	0.439
2007	dead	Haast	male	9	0.057
2007	dead	HariHari	male	24	0.676
2007	dead	Harihari	male	23	0.865
2007	dead	HariHari	male	18	0.822
2007	dead	Haast	female	9	0.159
2007	dead	Haast	male	8	0.175
2007	dead	Haast	female	6	0.360
2007	dead	Haast	female	8	0.456

Appendix 4

Supplementary material for chapter 6: Model code

```
% Variables

G = 50; % number of generations
S = 5000; % number of pairs, or half population size
SP= 0.95; % proportion in population of species 1 (represented as 0)
GD= 10; % genetic distance up to which animals accept each other as conspecific, in
percent
LH= 0.25; % likelihood of accepting a different species partner
R1= 9.4; % mean reproductive output species 1
R2= 11; % mean reproductive output species 2
V= 2; % variance of reproductive output
TSS=30; % no negative fitness effect up to this percentage of foreign genes


% loop it as often as required
for i=1:5 %500


% define start population
% two lines of size S, one representing females, one representing males. Genetic values
are 100 (one species, 100% genes of this species) or 0 (other species, 0% genes of the
first species) in the approximate proportions specified, where positions are determined
randomly.

% generates a row of the length of 2*S (population size) of 100 or 0. 100 represents
100% of genes of one species, 0 represents 0% of genes from that species, i.e. pure
individuals of the other species.
% the proportion of each species in the population is determined by SP. Entries are 0 if a
random number is smaller than the threshold specified (SP), otherwise it will be 100.
% half the values are stored in F, representing the females of the population
% the other half the values are stored in M, representing the males of the population

for k=1:S*2
if rand<SP
K(k)= 0;
else
K(k)= 100;
end
end

F(1:S)=K(1:S);
```

```
M(1:S)=K(S+1:S*2);

% Stores result as the first line of R (result)

R(1,:)=K;
count=0;

% Generation number
% Loops entire program from pair formation to next generation inclusive, G (generation
number) times, making sure the new F and M at the end of each iteration are used as
starting point for the next time.

for g = 1:G

% Pair formation
% The first number of F and first number of M are defined to be a pair, as are the second
of each, third of each, etc. Whether a pair reproduces or not is determined next.
% The critieria for accepting partners (which also define sexual attractiveness of hybrids
and ‘pure’ individuals for each other) could be changed at will for other species

% D is the genetic difference between the male and female of a pair.
% Partners always accept each other if their genetic difference (D) is below the specified
threshold value (GD). If they are more dissimilar they only accept each other with the
specified probability (LH)
% if the difference between female and male genetic values is smaller than the specified
GD (difference for accepting a partner), B=1 (i.e. the pair is a breeding pair).
% otherwise if a random number is lower than the specified LH (likelihood of accepting a
heterospecific partner) B=1 (i.e. is a breeding pair)
% otherwise B=0 (i.e. not a breeding pair)

%D=abs(F-M);

%for k=1:length(D)
%if D (k) < GD
%B(k)=1;
%elseif rand<LH
%B(k)=1;
%else
%B(k)=0;
%end
%end

% D is the genetic difference of potential partners, female - male. The
% normal curve describing the chance of such pairs to stay together has
% been defined by PopMean and StandDeviat.
% for each pairs differences, they have a chance of staying together
```

```
% determined by that distribution (via probabilitydensistyfunction)
% if they stay together, B=1 (ie they are a breeding pair), if not B=0

PopMean=-4.545;
StandDeviat=35.56;

D=F-M;
for k=1:length(D)
if rand <
normpdf(D(k),PopMean,StandDeviat)/normpdf(PopMean,PopMean,StandDeviat);
B(k)=1;
else
B(k)=0;
end
end


% in order to give rare species a realistic chance of finding a partner, all non-paired
individuals get a second try (and later a third)
% f and m represent all F and M values of paired (B nonzero) individuals
% F and M are as yet unpaired individuals (X is the opposite of B)

f=F(find(B));
m=M(find(B));

X=B-1;
X=abs(X);
F=F(find(X));
M=M(find(X));

% if f (and m) are smaller than S, that is if not all individuals are paired, the values of
unpaired males M are flipped to give different partners as before
% then the pair formation part is rerun, and f and m and F and M are updated

if length(f)<S
clear B D X

M=fliplr(M);

D=F-M;
for k=1:length(D)
if rand <
normpdf(D(k),PopMean,StandDeviat)/normpdf(PopMean,PopMean,StandDeviat);
B(k)=1;
else
B(k)=0;
```

```
end
end

%D=abs(F-M);

%for k=1:length(D)
%if D (k) < GD
%B(k)=1;
%elseif rand<LH
%B(k)=1;
%else
%B(k)=0;
%end
%end

f(length(f)+1:length(f)+nnz(B))=F(find(B));
m(length(m)+1:length(m)+nnz(B))=M(find(B));

X=B-1;
X=abs(X);
F=F(find(X));
M=M(find(X));

end
```

% A third chance can be given, again if not all pairs are mated now. The process is identical to the one before apart from the mixing procedure, which now does not flip but shifts all values by one (the first goes to the end)

```
if length(f)<S
clear B D X

M(length(M)+1)=M(1);
M=M(2:length(M));

D=F-M;
for k=1:length(D)
if rand <
normpdf(D(k),PopMean,StandDeviat)/normpdf(PopMean,PopMean,StandDeviat);
B(k)=1;
else
B(k)=0;
end
end

%D=abs(F-M);
```

```
%for k=1:length(D)
%if D (k) < GD
%B(k)=1;
%elseif rand<LH
%B(k)=1;
%else
%B(k)=0;
%end
%end

end
```

% all females are again combined in F, first those unpaired or paired from the last run, then those paired and stored in f. The same for males.
% B likewise first has the 0 or 1 values of the unpaired, then the rest is made up of 1s for the paired males and females

```
F(length(F)+1:S)=f;
M(length(M)+1:S)=m;

B(length(B)+1:S)=1;
```

% records number of nonbreeders

```
NB(1:G)=length(B)-nnz(B);
```

% reproductive output
% N determines the number of offspring of each pair based on the differential reproductive output of both species, based on genetics of female

% if B (breeding) =1 (i.e. the pair is a breeding pair), one of two formulas to find N are used. Which one is used depends on which species' mean breeding success (R1 or R2) is higher (the equations differ only in a plus or minus sign).
% The formula specifying offspring number N consists of a random number (normally distributed with mean 0 and variance1) multiplied with the variance (V) to give a stochastic element, added to a mean, which is calculated as the lower species' mean plus some extra mean proportionate to the genes of the female (i.e. a female exactly half way between both species also has a mean of offspring in the middle of both species' means)
% if B (breeding)=0 (i.e. the pair is not a breeding pair), the offspring number is 0

```
for k=1:S
if B(k)==1
if R1>=R2
N(k)=round(randn.*V+R1-(F(k).*abs(R1-R2)./100));
else
```

```
N(k)=round(randn.*V+R1+(F(k).*abs(R1-R2)./100));
end
else
N(k)=0;
end
end

% should N be a negative number, this really should be a 0 (less than no young are not
possible)

for k=1:S
if N(k)<0
N(k)=0;
end
end

% the genetic value of the young (Y) is determined as half that of the combined values of
the parents

Y = (F+ M)./2;


% Survival
% determines mortality of young based on their genetics

% clearing the old values ensures no values from the previous run are used, even if there
were more young than in the current run

clear O Z T A r s I

% determines a survival probability, L, based on criteria specified. This section can be
remodeled for each case as wished. Any number of intervals and threshold values could
be chosen and programmed.
% here: if the genetic value of young (Y) is larger than a specified value, L (the
likelihood of death) = T1 (a specified threshold value, e. g. if set to 0.4, 40% of young
live, so it specified survival not mortality),
% otherwise if Y is larger than the next specified value, L =T2
% otherwise L =T3

for k=1:S
if Y(k) < TSS
L(k) = 0.43; %T1=0.43
elseif Y(k)< (100-TSS)
L(k)= 0.43; %T2=0.43
else
L(k)= 0.43; %T3=0.43
```

```
end
end

% uses the survival probability L to determine which young survive.
% this needs to be repeated for each young, so N times per column of Y

% determine a vector a of length S, in which each number is the cumulative sum of all N
(number of young) values before
% this vector gets an additional 0 at the beginning, is now S+1 long

for k=1:S
a(k)=sum(N(1:k));
end

b=[0,a];

% O (offspring) lists each value Y (genetics of young) N (number of young) times, so
each young is now represented independently

for k=1:S
O(b(k)+1:b(k+1))=Y(k);
end

% the values of L are arranged in Z as the values of Y are in O, so that each genetic value
of a young has the corresponding likelihood of survival

for k=1:S
Z(b(k)+1:b(k+1))=L(k);
end

% creates T, which is 1 if a young survives, and 0 if it dies. This is a random decision
using likelihood Z.

for k=1:length(O)
if rand<Z(k)
T(k)= 1;
else
T(k)= 0;
end
end

% to prevent zero genetic values from being eliminated, 1 is added to O (it will be
subtracted again at a later stage). O is then multiplied with T, so that all values of dead
young become 0, but all surviving young retain their genetic values (plus 1). A is then
only the non zero values of A, and is reshaped to be one long vector.
```

```
A=(O+1).*T;
A=nonzeros(A);
A=reshape(A,1,length(A));


% Next Generation
% reduces the number of pairs randomly to the original population size

% a vector r of length A is generated, which contains random numbers
% using the values of r sorted for size, the 2*S value is determined. Values up to this are
included to give exactly 2*S individuals for the next generation.

r=rand(1,length(A));
s=sort(r);
C=s(2.*S);

% determines that if a random number is lower than C, the corresponding individual in A
is selected to go on to form the next generation (now I), values with too high r values are
set to 0, and eliminated.

for k=1:length(A)
if r(k)<=C
I(k)=A(k);
else I(k)=0;
end
end

I=nonzeros(I);

% the extra one added at the beginning of the survival process is subtracted again

I=I-1;

% to mix the values, they are formed to a two row matrix, which is rotated, and then
reshaped to one large vector. This is repeated. This process is deterministic but ensures
some mixing to prevent one side of the vector behaving differently from the other.

I=reshape(I,2,S);
I=rot90(I);
I=reshape(I,1,2.*S);
I=reshape(I,2,S);
I=rot90(I);
I=reshape(I,1,2.*S);

%This vector is then divided into two lines, F and M. This will overwrite the previous F
and M!
```

```
F=I(1:S);
M=I(S+1:2.*S);

% Result storage
%Store results in a row of R. This is NOT to be overwritten in the next loop.

R(g+1,:)=I;
average(g)=mean(R(g,:));
STD(g)=std(R(g,:));


% Generation number

end

% store data to be kept
% first, middle, final line of each? each 4000 values! would be times3
% times500... NO lines can be kept, average and STD only

average1(i)=average(1);
average5(i)=average(5);
average10(i)=average(10);
average15(i)=average(15);
average20(i)=average(20);
average25(i)=average(25);
average30(i)=average(30);
average35(i)=average(35);
average40(i)=average(40);
average45(i)=average(45);
average50(i)=average(50);
STD1(i)=STD(1);
STD5(i)=STD(5);
STD10(i)=STD(10);
STD15(i)=STD(15);
STD20(i)=STD(20);
STD25(i)=STD(25);
STD30(i)=STD(30);
STD35(i)=STD(35);
STD40(i)=STD(40);
STD45(i)=STD(45);
STD50(i)=STD(50);

% finish repeats
end
```